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SYMBIONTICISM
AND
THE ORIGIN OF SPECIES

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AND
THE ORIGIN OF SPECIES

BY
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To

JOHAN AUGUST UDDEN

My First Teacher In Biology,
This Book Is Affectionately
Dedicated

PREFACE

The study of mitochondria in recent years has come to occupy an important position in biology. Two points of view are held in regard to the nature of these microscopic structures, and this circumstance appears to be responsible for the diversity of opinion as to the activities of these bodies. The most commonly accepted view holds that mitochondria are cell-organs derived from the cytoplasm. The other view, that they are microörganisms "symbiotically" united to the cell, has attracted only a few adherents, and apparently has been looked upon as a fantastic and improbable theory.

The bare statement that all living cells of plants and animals contain small bodies that are independent entities capable of a free existence, or in other words, that all cells contain bacteria or microörganisms, is, perhaps, a little shocking to those who hold dogmatic ideas on cell physiology. The recent advances in physical chemistry also have served to direct our thoughts and notions on "life-organization" into channels which would belittle the importance of "life" in the organization of protoplasm. Obviously, physical and chemical forces play a rôle of fundamental importance in the morphology and physiology of living organisms, but we are still groping in ignorance as to the real nature of living matter, and we are not at liberty to forget or belittle the significance of "life" in the analyses of living bodies.

During the past seven years the author has investigated the nature of mitochondria, and has arrived at the unqualified conviction that these bodies in the cell are bacterial in nature. The evidence for this conclusion has been published in a series of papers. It has been evident that a large

number of biologists have been skeptical of the results obtained in these investigations, and it also appears that many have been opposed to the fundamental conception that mitochondria are bacteria. It was these circumstances that urged the author to seek a rational hypothesis whereby the significance of microörganisms in the cell could be explained. A study of the literature on "micro-symbiosis" revealed a wealth of evidence that supports and emphasizes the significance of bacteria in the origin of species.

A principle that is so revolutionary as Symbionticism, not only introduces a new principle in organic evolution, but also inserts a new conception in heredity, development and cell-structure. While it may not be "good scientific form" to indulge in speculative hypotheses, the new viewpoint affects the hypotheses and theories that are held at the present time in major divisions of biology, and the author has deemed it advisable to discuss some of these problems. Obviously it would be most unusual if all the hypotheses that are advanced in this book should in the end prove to be correct. They are based upon *some* evidence which in many instances is scanty, but in the present state of knowledge it represents the only evidence available. It is obvious that further and more extensive researches on the particular problems may result in modifications and the development of new hypotheses. These theories have been included in this book mainly to indicate the feasibility of Symbionticism, and to stimulate further researches on these fundamental problems.

It is pertinent to emphasize that the chief object of this book is to summarize the evidence for the bacterial nature of mitochondria, and to present some of the evidence that demonstrates the fundamental rôle played by bacteria in the origin of species.

The search for evidence bearing on the theory has carried me into many fields of biological science as well as into the

domains of chemistry and physics. It is an impossible task for any one individual to cover thoroughly the literature even in one of these fields of research, and I must beg the indulgence of my critics in this matter. In my desire to submit these studies in a small and concentrated volume, I have passed over or mentioned only briefly many deserving and creditable pieces of investigation. Obviously, some researches of importance may not have come to my notice.

I have made extensive use of Professor Paul Buchner's monograph "Tier und Pflanze in Intra-zellularer Symbiose." This comprehensive review of the literature on "micro-symbiosis" has shortened my labors by many months. The extensive treatise by Professor Edmund B. Wilson in his recent revision of "The Cell in Development and Heredity," Professor E. G. Conklin's lectures on "The Mechanism of Evolution," Professor Vernon L. Kellogg's "Darwinism Today," Professor H. F. Osborn's "The Origin and Evolution of Life," and many other treatises mentioned in the text have served as short-cuts to a better appreciation of the accomplishments in various biological fields. I gratefully acknowledge my indebtedness to these valuable works.

To Professor Arthur I. Kendall, Doctor F. Löhnis and Doctor Montrose T. Burrows, I am especially indebted for sympathetic interest and encouragement in my investigations and for many valuable suggestions. To my colleague Doctor Ralph G. Mills I am indebted for painstaking criticisms of the manuscript. I am, also, greatly indebted to other colleagues at the University of Colorado School of Medicine for opportunities to discuss various phases of my investigations and hypotheses. To my technicians, Mr. Ivan M. Way and Miss Josephine Jones, I am especially grateful for conscientious and reliable assistance in the technical work of the researches.

It is a great pleasure to acknowledge my indebtedness to Mrs. Henry S. Denison for research funds which were

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Lastly, it is a genuine pleasure to acknowledge my indebtedness to the publishers, The Williams & Wilkins Company, for assuming the responsibility to publish this book, and for pleasant and courteous dealings.

IVAN E. WALLIN.

University of Colorado School of Medicine,
Denver, August 18, 1926.

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CHAPTER I

Introduction

The earliest indications of a recognition of the essential principle embodied in the theory of organic evolution—that the more complex organisms have evolved from simpler—is, perhaps, to be found in the writings of ancient scholars. These early writings do not state a definitely formulated theory of evolution, but they contain hazy references that similar forms of life may have had a relationship in origin. Such deductions were reasonable conclusions drawn from observations of the more superficial resemblances of many animal groups. Since these earlier times, the study of plants and animals has developed into two major fields of scientific research, botany and zoology, each with a number of distinct subdivisions. During the development of these sciences, particularly zoology, there gradually crystallized a conception of organic evolution. The similarity of animals was found to be not only superficial, but the minute structural details exhibited a close resemblance in large groups of animals. Through a large series of observations and researches by a host of pioneer investigators, data accumulated which prepared a firm foundation for the theory of organic evolution. Although Linnaeus upheld the separate creation theory in the origin of species, the introduction of his binomial system of nomenclature served in great measure to emphasize the relationships of plants and animals. It was perhaps Lamarck who first clearly stated the theory of organic evolution as we understand it today.

In the earlier stages of the postulated theory, philosophic speculation occupied the center of the stage. Nevertheless, we gained the principles of "The Struggle for Existence" and

"The Survival of the Fittest" from these earlier observations and debates. To Darwin we are indebted for a lasting demonstration of the method of scientific procedure, and for the first extensive attempt to analyze the factors involved in organic evolution. To Darwin, also, is due the credit for the first comprehensive and acceptable attempt to explain the manner of the origin of species.

The extensive investigations in all departments of biology, the critical analyses and the illuminating discussions which have followed in the wake of Darwin's dissertations, have served to clarify our conceptions and to crystallize the problems involved in organic evolution. It has become apparent that evolution is an exceedingly complex process, dependent upon a great number of factors. There appear to be three major features of organic evolution controlled by three cardinal principles. The concensus of opinion of biologists today credits Darwin with having established one of these principles. The other two principles remain to be established. Besides these three cardinal principles, there apparently are a great number of factors influencing the operations of the major principles. These factors are the various influences that have been long recognized as the modifiers of protoplasmic reactions.

What then are the three major features of organic evolution? A clear and concise statement of these features is not found in the literature, so it becomes necessary to attempt to formulate it here. The conception of organic evolution embodies, first of all, a conception of the origin of new forms. This conception is embodied in the term "origin of species." This, however, is not the sum total of organic evolution. It is well known that along with the ever increasing number of kinds of plants and animals, many that were abundant in earlier geologic times have since disappeared; other forms, apparently, are disappearing today. The kaleidoscopic character of life upon the earth

is the second major feature of organic evolution and, apparently, is controlled by a separate principle. This principle operates to control the ultimate character of the organisms present in any stage of the progress of evolution; in other words, it determines the retention or destruction of species on the earth. The third major feature of organic evolution is concerned with direction or progress. This feature has been spoken of as the principle of progressive evolution. Biologists are quite agreed that there is a "something" directing organic evolution toward an ever more complex and specialized end. There are many examples of individual groups (genera) of animals which do not follow the general law, but exhibit regressive characters. However, regressive forms are explainable exceptions, and do not destroy the general principle of progressive evolution.

A great amount of confusion has existed in the ranks of the evolutionists. This confusion, it appears, has been largely due to a lack of proper evaluation of the cardinal features involved in organic evolution. The discussions have centered chiefly about Darwin's explanations. In Natural Selection, Darwin has established one of the cardinal principles that is operative. Natural Selection determines the character of life at any period in evolution; it is the principle that controls the retention or destruction of formed species. Darwin, apparently, recognized the insufficiency of Natural Selection to produce new species and introduced other factors to fill this gap in the explanation of organic evolution. These subsidiary factors have not been acceptable to a large group of biologists. Modern writers have recognized the insufficiency of Darwin's hypothesis to explain the origin of species. The "unknown factor" in organic evolution has been especially emphasized by Osborne, Bateson, Kellog, and other recent writers. This "unknown factor" is especially concerned with the origin

of species. It is the factor that causes permanent variations in the structure of animals and plants.

Another unknown factor of evolution is the principle determining the direction of organic evolution, or the goal toward which evolution is proceeding. This factor has

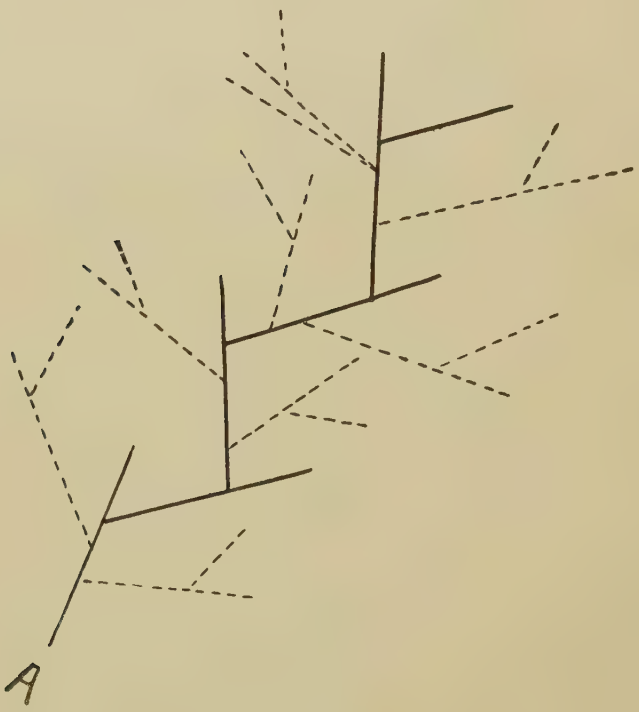


Fig. 1

been recognized, but very little has been attempted or accomplished in determining its nature. It is possible that this particular feature of organic evolution has been and still is a stumbling block to the layman's appreciation of the reality of organic evolution. From a common ancestor

various diverging stocks have arisen, but only one stock forms the channel through which organic evolution proceeds. The diagram in figure 1 may serve to make the principle more clear. The various lines both solid and dotted represent new species that have arisen from the common stock A. The dotted lines represent species that have arisen but are off of the line of progressive evolution. These forms may persist for longer or shorter periods and may give rise to new and more complicated species, but they are not in the direct line of evolution and therefore are not enduring. The solid lines of the diagram represent the species which are in the line of progressive evolution.

The question arises: why has organic evolution proceeded along the lines that it, apparently, has chosen? The factors involved in Natural Selection may account for the ultimate destruction of the aberrant species and stocks, but is Natural Selection sufficient to determine the course that organic evolution has taken? Does Natural Selection supply an element of progress, a something that has directed evolution to a more and more complex end? This is a mooted point, and it appears to me, as it has to many others, that Natural Selection, by itself, is not sufficient to determine the direction of organic evolution. If we accept Natural Selection as the cardinal principle controlling the direction of organic evolution, then it is necessary to recognize a preordained purposive factor in Natural Selection that carries us beyond our powers of comprehension. Natural Selection can only deal with that which has been formed; it has no creative powers. Any directing influence that Natural Selection may have in organic evolution, must, in the nature of the process, be secondary to some other unknown factor.

In attempting to determine the cardinal principle involved in the origin of species, it appears reasonable to assume that a number of factors combine to inaugurate those changes in an organism that shall lead to the origin of new species.

Of all the factors that play a rôle in such a process, one factor, whatever it may be, is crucial or fundamental. The remaining factors, obviously, would occupy varying degrees of importance in the completion of the process. The fundamental factor in the origin of chemical compounds consists of the presence of two or more chemicals in close spacial relationship. In order that a chemical reaction, resulting in a new compound, shall take place it is also necessary that various extrinsic factors be properly satisfied. Obviously, these extrinsic factors are not necessarily the same for different chemical combinations, but the fundamental factor, two or more chemicals in close proximity, is invariably constant. Exceptions to these statements, apparently, may be found in the inorganic world. It has been claimed that it is possible to produce a more complex molecule from a simpler one by the introduction of extrinsic factors alone. It is possible, also, that such a process has been operative in inorganic evolution, but, if so, it must be emphasized that this is the exception and not the rule. In our experimental laboratories, as well as in nature's laboratory, the presence of two or more chemicals appears to be essential in the production of a new and more complex molecule. Does the origin of species differ fundamentally from the origin of chemical compounds? Are there fundamental factors and principles utilized in organic evolution which are absent in inorganic evolution? In recent years we have come to realize that the gap between the organic and the inorganic world is narrowing and it appears that the time is not far distant when it may completely disappear. Those activities and properties which only a few years ago were classified as "vitalistic" are today recognized as but the ultimate expression of chemical and physical forces. The vitalistic school, which at one time dominated biological thought, has all but completely disappeared. There is no evidence at hand to indicate that organic evolution is

fundamentally different from inorganic evolution. Inorganic chemical reactions are dependent on the same set of extrinsic factors that govern organic chemical reactions. It appears, also, that the fundamental principle involved in the origin of species is primarily of the same general nature as it is in the origin of chemical compounds. Thus, just as it is necessary for two or more chemical compounds to be brought together if a new compound is to be formed, it may be necessary for two or more forms of life to unite in the production of a new species.

While we lack specific data on some phases of plant and animal behavior, it appears from general observations, that higher plants and animals exhibit a greater degree of stability than do the lower forms. The lowliest forms of life—the bacteria—appear to exhibit the greatest degree of variability. These lowly forms which, apparently, are neither plant nor animal, appear to respond more readily to environment than do the complex forms of life. It has been suggested, and it appears likely, that the bacteria are the primordial organisms from which all higher organisms, both plant and animal, have sprung.

The nature of the process by which this phase of organic evolution may have occurred is indicated in the phenomenon symbiosis. Notwithstanding the fact that symbiotic relationships have been studied for many years, the fundamental principle embodied in the phenomenon does not appear to have been recognized. This, probably, is due to the fact that the first recognized examples of symbiosis dealt with those cases in which both symbionts were complex animals or plants, and the life relationship was looked upon more in the nature of a biological curiosity, than the expression of a fundamental principle. A more universal type of symbiosis is represented in those relationships in which one symbiont consists of a microörganism. It is this latter type which may be designated "microsymbiosis" that appears to offer the solution to the problem of the origin of species.

Microsymbiosis appears to be a universal biological phenomenon. The cells of all normal plants and animals contain minute bodies which have been named "mitochondria." In a series of researches the author has been able to demonstrate the bacterial nature of these bodies. Their universal presence in the cell coupled with the known properties of bacteria, appear to indicate that mitochondria represent the end adjustment of a fundamental biologic process. The establishment of intimate microsymbiotic complexes has been designated "Symbionticism" by the author. Symbionticism, then, is proposed as the fundamental factor or the cardinal principle involved in the origin of species.

It is a rather startling proposal that bacteria, the organisms which are popularly associated with disease, may represent the fundamental causative factor in the origin of species. Evidence of the constructive activities of bacteria has been at hand for many years, but popular conceptions of bacteria have been colored chiefly by their destructive activities as represented in disease. This destructive conception has become so fixed in the popular mind that the average person considers bacteria and disease as synonymous. Bacteria occupy a fundamental position in the world as it is constituted today. It is impossible to conceive of this world as existing without bacteria. The cyclic rotation of essential food elements is dependent on bacterial activity. Thus, fixed nitrogen which is practically limited in amount in the world and is, also, the pivotal element in living matter, is made available through bacterial activity. Those kinds of bacteria which produce disease in man and animals, the pathogenic bacteria, represent only a small fraction of all the known varieties of bacteria. The activities in nature that are dependent upon bacteria are numerous and diversified. It is this diversity of bacterial properties, that lends support to the theory of Symbionticism as the causative factor in the origin of species.

The basis for the postulation of the theory of Symbionticism rests upon the nature of mitochondria. Our first task, then, in attempting to establish the theory consists in reviewing the evidence for the bacterial nature of mitochondria. It also becomes necessary to seek in nature for further evidence of the manifestations of the principle, for, if mitochondria represent the end adjustment of a biologic process, then it appears that it should be possible to find in nature examples representing various phases of this manifestation in actual operation. A large number of such examples have been described in biological literature. The morphologic and physiologic responses exhibited in many of these symbiotic associations furnish concrete evidence of the manner in which the principles of Symbionticism may be operating in the origin of species.

CHAPTER II

History of Mitochondrial Research

Interest in mitochondria dates back to 1890 when Altmann published his treatise on "The elementary organisms and their relationship to the cell." Earlier observers had recognized granulations in the cell, but the presence of these granules was only occasionally observed and consequently suggested bacterial contamination. The special methods of fixation and staining developed by Flemming, Altmann, and Benda made it possible to demonstrate these minute bodies consistently. This ubiquity of the granules in the cells of plants and animals led Altmann to formulate an hypothesis concerning their nature and significance in relation to the cells of all organisms. Altmann named these granules "bioblasts" and believed that they represent the ultimate units of living matter, and that the cells in which they are situated are otherwise lifeless passive elements. This revolutionary conception of the cell was attacked by contemporary investigators (Verworn, and others) who clearly showed that Altmann's conception was erroneous. Along with the rejection of this conception of the cell, the idea of the "bioblast" as the elementary or primordial organism was also rejected.

While Altmann's conception of the nature of these cell granules was not accepted, his work served to stimulate an active and increasing interest in these structures. The constant presence of the granules in the cell pointed strongly to some significant functional relationship. With a few exceptions which shall be discussed later, the granules came to be looked upon as originating from the cytoplasm and consequently genetically a part of it. The extensive re-

searches that followed Altmann's postulation of the "Bioblast Theory" have brought to light many interesting and instructive activities and properties of these granules.

The granules have been given various names, but recently the term "mitochondria" suggested by Benda has come into general usage, although the term "chondriosome" is used extensively. Mitochondria have various shapes, globular, rod-like, filamentous and irregular. Various attempts have been made to classify mitochondria according to their shape, but as E. V. Cowdry ('18) has intimated in his extensive review of mitochondrial literature, the shape of mitochondria is of no particular significance. These classifications have not entered into common usage. With only a few exceptions, mitochondria have been demonstrated in all groups of plants and animals. It appears probable that when a proper technique is used there will be no exceptions.

It is not the purpose of this historical sketch to give a detailed account of the literature on mitochondria. The interested reader who desires to follow this in greater detail is referred to Duesberg's review of the literature up to the year 1912 (in German) and to Cowdry's review up to the year 1918. The sketch given here is selective and mainly intended to emphasize the salient features of mitochondria that have been advanced in the literature, and which have a direct bearing upon the principles of Symbionticism.

A number of significant features and properties of mitochondria have been brought out in the literature since the pioneer researches of Altmann. In 1897 Benda studied the formation of the spiral filament in the spermatozoa of the mouse and found that mitochondria are transformed into this structure. His observations have been confirmed by a number of investigators. Meves ('12), Held ('12), Romeis ('13), Fauré-Fremiet ('13), Duesberg ('15), Levi and others have observed that the mitochondria or their products enter the egg with the sperm on fertilization. On the basis of

these observations the idea was advanced that mitochondria play a rôle in heredity. Meves in particular has advanced this theory. This conception finds a certain amount of support in the views held by Wilson and Conklin who recognize that the cytoplasm may play a rôle in hereditary transmission.

In 1894 Altmann expounded the doctrine "Omne granulum ex granulo" fashioned after the well-known aphorism of Virchow: "omnis cellula e cellula." Numerous studies have since been made in an attempt to determine the manner in which mitochondria increase in the cell. Duesberg, Meves and many others have observed what they regard as mitochondrial division, and believe with Guilliermond that "the mitochondria result from the division of the preëxisting mitochondria of the egg, and that none of them arise 'de novo' in the cytoplasm." Chambers ('15), on the other hand, observed that mitochondria suddenly appear in the cytoplasm and is against the idea of "mitochondrial continuity." It must be emphasized that other granules besides mitochondria are supposed to be present in the cytoplasm in many instances. While there is no occasion to question Chambers' observations, there nevertheless remain various possibilities in the interpretation of the phenomenon. It is possible that the granules which were observed to arise "de novo" in the cytoplasm have no relationship with the mitochondria. Cowdry has found that the mitochondria sometimes go into "solution" in the cell. My own observations (Wallin, '22) on the mitochondria of lymphocytes, confirm Cowdry's observation. Under certain conditions of the cytoplasm, whatever they may be, mitochondria are miscible with the cell cytoplasm. In such cases the mitochondrial dye stains the entire cytoplasm. In fixed material, obviously, it is impossible to determine if the mitochondrial material could again collect into granules. Chambers' observation on the living cell

would tend to show that such may be the case. In a changing chemical system such as cytoplasm is, one may expect solution and precipitation phenomena to occur. This is in agreement with observed phenomena in colloidal chemistry. There is no available evidence to show that a "solution" and "precipitation" phenomenon in connection with mitochondrial material would necessarily exclude the possibility of division by fission when this material is in the physical state of granules or bodies. Cowdry is inclined to believe that true mitochondria arise *de novo* in the cytoplasm. He bases his opinion on the assumption that mitochondria have a "phospholipin-albumin" composition, and that it does not seem reasonable that such a complex would actively divide like microörganisms.

The consensus of opinion of modern cytologists, as well as recent researches (Horning, '25; Takagi, '25; Causey, '25; Horning, '26; and others) favor the idea of mitochondrial continuity.

In 1899 Benda investigated the mitochondria of the developing muscle cell, and believed that the mitochondria differentiate into the myofibrils of the muscle fibre. Meves later arrived at the same conclusion. Duesberg in 1909 and 1910 conducted extensive investigations of the subject. He was able to follow the transformation changes and noted the diminution of mitochondria parallel with the increase in fibrils. The conclusions arrived at by Benda, Meves, and Duesberg have been confirmed by von Kurkiewicz, Leplat ('12), Levitsky ('10) Favre et Regaud ('10), Hoven ('10), Prenant ('11), Arnold ('12), Schäfer ('12), Luna ('13), Brück ('14) and many others. Against this conception of the nature of myofibril-formation are the opinions of Heidenhain ('11), Levi ('11), Gurevitsch ('13) and Cowdry. Cowdry bases his opinion on some slight variations of staining in his preparations of chick embryos, and on the impossibility of mitochondria, assumed to be composed of phospholipin-

albumin, being transformed into myofibrils which contain tyrosin.

The conceptions embodied in Benda's work that mitochondria enter into histogenetic differentiations, apparently, stimulated a number of investigators to study mitochondria in relation to various histogenetic activities. This point of view was further strengthened by Regaud's "eclectosome theory," which held that mitochondria behave somewhat like plasts, choosing and selecting substances from the surrounding cytoplasm, and transforming them into diverse products. Meves, in 1910, investigated the origin of connective tissue fibrils. He found that mitochondria leave the cell and become filamentous, and he believed that they enter into the formation of the fibrils. Against this conception is, apparently, the work of M. R. Lewis ('17) who studied connective tissue fibril formation in tissue cultures. Cowdry criticizes Meves' work from two points of view. He assumes that the chemical composition of the mitochondria of all cells is the same, and that the assumed phospholipin-albumin structure is incompatible with collagen fibril formation. On the further assumption that the mitochondria of all cells are alike and the fact that filamentous or thread-like mitochondria are present in cells which do not contain fibrils, Cowdry holds that the development of filamentous mitochondria in Meves' preparations have no significance.

Firket ('11) investigated the origin of epidermal fibrils and concluded that they arise from the differentiations of mitochondria. Duesberg later confirmed this work of Firket. Meves, in 1907, investigated the origin of fibrils in nervous tissue. He concluded that the mitochondria of the nerve cells differentiate into the neurofibrils. A number of investigators have also confirmed his work, of whom we might mention Hoven and Arnold. Marcora, Levi, and Gurevitsch, on the other hand, reject the conception that neurofibrils are formed from mitochondria.

The investigations of Regaud, Fauré-Fremiet, Löwschin and others on the chemical nature of mitochondria cast a cloud of doubt on the "diverse transformation activities" that had been attributed to mitochondria. Thus, Cowdry ('18), in summing up his criticism on fibril formation, expresses the sentiments of cytologists in general when he remarks "that it is somewhat illogical to suppose that substances of such diverse chemical constitution are all formed through the transformation of a single substance, a phospholipin, combined perhaps with a small fraction of albumin."

The influence that these chemical investigations have had on subsequent mitochondrial research has been unfortunate. It is pertinent to analyze minutely the evidence on which the ideas on the chemical constitution of mitochondria have been based. This may be facilitated by considering the collected evidence as it is given by Cowdry in his critical review of the literature. After pointing out that three individuals, Regaud, Fauré-Fremiet and Löwschin arrived at the same conclusion independently, he tabulates the evidence briefly as follows:

(1) Mitochondria are almost completely soluble in alcohol, chloroform, ether, and dilute acetic acid. They are rendered insoluble by chromization. They are not doubly refractile and they do not stain with either Sudan III or Scharlach R. They are only sometimes blackened with osmic acid.

From the above evidence, apparently, it is assumed that because mitochondria in general are sensitive to these chemicals they all have a like constitution. However, it is well known that mitochondria vary in chemical constitution at different periods in the life of the cell. Thus, to quote from another section of Cowdry's treatise, "It is well known that the mitochondria undergo definite chemical changes in the course of spermatogenesis. Regaud (1910, p. 294) clearly showed that their resistance to acetic acid grows

greater and greater; in fact, the structures which they form, nebenkern and spiral filament, were known and recognized long before the mitochondria in the earlier stages of spermatogenesis were brought to light." Assuming that all mitochondria responded in a like manner to these various chemicals this would not be sufficient evidence to establish a more or less definite chemical constitution of these microscopic bodies. In some of my own experiments (Wallin '22a), various bacteria were subjected to these chemicals. It was found that some strains of bacteria responded in a manner similar to the usual response of mitochondria. Bowen's extensive researches on the nebenkern of certain insects demonstrated that chemical transformations occur in the mitochondrial body during its complicated changes. These chemical variations become apparent in the staining reactions.

(2) It is said that part of the mitochondrial substance is not soluble in these fat solvents and it is supposed that this portion is albumin (see also Bullard, 1916, p. 26), for formalin and bichromate, which are used as fixatives for mitochondria, are energetic coagulants of albumin. Millon's reagent is the only color-test for protein which can be satisfactorily applied to material in section (the xanthoproteic reaction may also be used, but it is less satisfactory because it is more destructive). I learn from Dr. R. R. Bensley that the mitochondria do not give a definitely positive Millon reaction in comparison with the strong Millon reaction which is given by such cytoplasmic structures as the zymogen granules. Even if there were a change in color in the mitochondria it might not be of sufficient intensity to be appreciated in filaments of such extreme fineness as mitochondria (0.2 micron in diameter) embedded in a colored cytoplasm. I have obtained no success with the xanthoproteic reaction. Mitochondria do not give any of the color reactions of polysaccharides.

This section, apparently, is intended to contain the evidence for the partial albumin constitution of mitochon-

dria. In the first sentence the word 'supposed' gives a clue to the finality of the evidence.

(3) Artificial mitochondria have been made by Löwschin of lecithin in different salt and albumin solutions (resulting in the formation of lecithalbumin), which apparently present the same form and solubilities as true mitochondria. They form granules, rods, and filaments which, he claims, multiply by division. He embedded them in glycerin-gelatin, fixed them, and found that they stained in the usual way by the various mitochondrial methods.

The production of rod-like, coccoid, and variously-shaped "precipitation bodies" in solutions is a common phenomenon in colloidal chemistry, and has been obtained in various ways with different kinds of reagents. Certainly this is not a phenomenon limited to solutions containing a phospholipin-albumin complex.

(4) The temperature solubility of mitochondria may also be significant. It has been discovered by Policard (1912d, p. 229) in the case of animal tissues, and by N. H. Cowdry (1917, p. 220) in plants, that the mitochondria are soluble at a temperature from 48° to 50°C., while the other parts of the cell remain practically unaffected. Phosphatids have a low melting-point also.

Because mitochondria disappear at a temperature of 48° to 50°C., and the melting point of phosphatids is also low, does not necessarily imply that the two are identical in constitution. In some experiments that the author (Wallin, '22a) made on the effects of temperature on various bacteria, it was found that some strains of bacteria "dissolved" at this temperature.

(5) Apparently the specific gravity of mitochondria is somewhat greater than protoplasm (Fauré-Fremiet, 1913, p. 602). This is determined by the centrifuge method. If they are thrown down they are said to be of high specific gravity. If the protoplasm is

in the physical condition of a "gel" rather than a "sol" as in the nerve cell, the distribution of the mitochondria is unaltered by centrifuging (Key). There is no reason to believe that the mitochondria themselves are different. At any rate, where the method is applicable (i.e., in egg cells) the mitochondria are heavier than protoplasm, in which respect they conform to what we know of phosphatids and differ sharply from oils and neutral fats, which rise to the surface and float instead of being thrown down.

Granting that the specific gravity of mitochondria is greater than that of the cytoplasm of the cell, such a circumstance would not constitute evidence that they are related to phosphatids merely because the latter are heavier than cytoplasm. Regards the probability that the mitochondria may be different in different varieties of cells, Cowdry ('18, p. 85) remarks "In some *varieties* of cells the constitution of the mitochondria apparently differs, slightly but noticeably, from that of the mitochondria in other cells, though in cells of the same kind their composition is very constant." The staining reactions of mitochondria when the Giemsa stain and the Kull method are used, demonstrate clearly that the mitochondria of a single cell are not all alike.

(6) Mitochondria act as solutes for various substances. They are often pigmented and assume the most brilliant hues. Prenant (Asvandourova, 1913, p. 293) has actually styled them "chromochondria" on this account. This solution of other materials in mitochondria is particularly frequent in plant cells. It may or it may not be significant from the point of view of their constitution.

The evidence here does not appear to have any specific bearing on a phospho-lipin-albumin constitution.

(7) There seems to be a certain correspondence between variations in the histological picture of mitochondria and the variations

in the phospholipin content of the same organ on chemical analysis. Thus Mayer, Rathery, and Schaeffer (1914, p. 612) have been able to alter the mitochondria experimentally in liver cells. In stages with more mitochondrial substance, chemical analysis showed an increase in phosphorized lipoid; in stages with less, a diminution. Fauré-Fremiet (1912b, p. 347) has extracted from the ovaries and testes of Ascaris a phosphatid with properties identical with those of mitochondria in the cells of these organs.

The evidence given here is limited in application. The experiments of Mayer, Rathery and Schaeffer were based on chemical analysis and, apparently, demonstrated that there was a relationship between the number of mitochondria and the amount of phosphorized lipoid present. This work was done on the liver. Is there any evidence that the same would hold true in other tissues? The evidence is not given. Furthermore, it is at least possible that the methods used to reduce the number of mitochondria at the same time affected the lipoid constituents of other portions of the cell. The work of Fauré-Fremiet also is not conclusive in that he did not demonstrate that the phosphatid which was extracted came from the mitochondria.

(8) Russo (1912, p. 215) has apparently been able to increase the number of mitochondria in the oocytes of the fowl by the injection of lecithin. R. Van der Stricht (1911, p. 455) found that there are two different kinds of eggs in the cat, one containing much vitellus and the other containing only a small amount; and further, that, following intraperitoneal injections of lecithin, the relative number of female offspring increased noticeably. In the normal condition 62 per cent are males, while after treating in this way only 23 per cent are males. That is to say, the administration of lecithin increases the amount of deutoplasm in the eggs, increases the number of eggs with much deutoplasm as contrasted with those with small amount, and in this way increases the percentage of females in the offspring. While this is of great interest in the determination of sex, and will be discussed in that

connection, it is also of importance as an indication of a possible relationship between the amount of mitochondria and the phosphatid lecithin.

The "evidence" here deals with possibilities and not with pertinent facts.

In summing up, we find that only one piece of investigation, that of Mayer, Rathery and Schaeffer has definite bearing on the problem. It relates only to the mitochondria of the cell of a single organ,—the liver. While we can neither deny, nor affirm the phospholipin-albumin constitution of mitochondria, there is no reason to conclude that mitochondria have the same constitution in all kinds of cells, and at all periods in the life of the cell. We certainly are not justified in rejecting the work that has been done on the histogenesis of fibrils on the basis of an incompatibility with the assumed chemical constitution of mitochondria. We must frankly confess that the chemical constitution of mitochondria is not known. It is interesting to note that Cowdry himself recognizes the variability of the chemical constitution of mitochondria, in other parts of his thesis (p. 85). He suggests that this variability may be more apparent than real: "it may be due to factors other than a difference in the mitochondria—to changes in the water content, for example." This, it appears to me, is a weak substitute for the rational interpretation suggested by such variations.

Mathews ('15), in his text book on Physiological Chemistry, states that the phospholipins or phosphatids are "among the most important substances in living matter. For they are found in all cells, and it is undoubtedly their function to produce with cholesterol, the peculiar semifluid, semisolid state of protoplasm. The latter holds much water in it, and does not dissolve. Indeed it might be said that the phosphatids with cholesterol make the essential physical substratum of living matter." Let us then assume that the

mitochondria are phosphatids, would this phosphatid constitution be incompatible with fibril formation? In the light of Mathews statement, and we have many reasons to accept the statement, mitochondria contain one of the essentials in the physical substratum of living matter. If we assume a phosphatid constitution of mitochondria then it certainly appears logical that mitochondria are protoplasmic in nature; in other words, they possess one of the essential components of living matter. If mitochondria are living structures, we would naturally expect them to exhibit differentitation into many and diverse structures. However, such argument based on assumptions is not sufficient to replace evidence gained by observation and experimentation.

Various other histogenetic activities have been attributed to mitochondria. Meves in 1908, stated that "with the specialization of the embryo into different organs and tissues, primitively similar cells assume special functions which find expression in characteristic structures or differentiations. All these products, no matter how heterogeneous they may be, arise through the metamorphosis of one and the same elementary plasma-constituent, the chondriosomes" (quoted from Cowdry). Meves' hypothetical statement has found a rather wide confirmation in the researches of subsequent investigators. The following list taken from that given by Cowdry will serve to indicate the range of these described activities: Anthocyanin (Guilliermond), Apparato reticulare (Hoven), Aqueous humor (Mawas), Batonnets of Heidenhain (Policard), Carotine (Guilliermond), Cerebrospinal fluid (Grynfelt and Euziere), Chlorophyl (Guilliermond), Chromoplasts (Guilliermond), Ciliary apparatus (Saguchi), Connective tissue cell granulations (Renant and Dubreuil), Granulations (Meves), Epidermal cell secretion (Saguchi), Fat (Altmann), Glycogen (Arnold, Alexieff), Goblet cell mucous (Grynfelt), Hemoglobin pigment (Ciaccio), Leuco-

plasts (Lewitsky), Lipoid in nerve cells (Cowdry), Liposomes (Fauré-Fremiet), Mammary gland secretion (Hoven), Melanin (Asavadourova), Mucorine (Moreau), Pancreatic zymogen (Hoven), Parathyroid secretion (Bobeau), Parotid secretion (Regaud and Mawas), Pigment, (Prenant), Poison-gland secretion (Torraca), Prepucial-gland secretion (Altmann), Prostate secretion (Akatsu), Retineal pigment (Luna), Sebaceous-gland secretion (Nicholas, Regaud and Favre), Starch (Guilliermond), Submaxillary gland secretion (Regaud and Mawas), Suprarenal pigment (Mulon), Suprarenal secretion (Mulon), Thyroid-gland secretion (Grynfelt), Tyrosin (Asavadourova), Urea in the kidney (Oliver), Venom (Grynfelt), Vitellus (Loyez), Yolk (Coghill). In many instances of the above named activities, the researches on which they have been based have been confirmed by other investigators, and in some instances the reverse has occurred.

It would be quite beyond the scope of this treatise to review and analyse all these investigations. However, the researches of Levitsky ('10), Pensa ('12), Guilliermond ('12a; '12b), and a host of other investigators on plast formation deserve more than passing notice. A number of varieties of plastids are found within the cells of plants. These plastids are concerned with the production of specific products in the plant. Guilliermond, in particular, has conducted extensive investigations on the origin of these plasts. He has apparently demonstrated the transformation of mitochondria into the various kinds of plastids. His work has been accepted in standard text books. Apparently, no serious refutation of Guilliermond's work has appeared. Cowdry is not inclined to accept these interpretations on plast formation, and states that "the work which has been done so far is extremely suggestive, but it is not conclusive." It appears that the *assumed* fixed phospholipin-albumin constitution of mitochondria has influenced Cowdry's criticisms. The *living* properties of

plasts has been emphasized in recent years, and has formed the basis for a pertinent and significant theory advanced by Merejkovsky in 1920. On the basis of the relationship of plant plastids to the independent plast-like organisms, the blue-green algae, Merejkovsky has advanced the theory that all the higher green plants are symbiotic complexes. A further consideration of Merejkovsky's theory will be given in a subsequent chapter.

The relationship of mitochondria to secretory processes has been investigated extensively. The results in these researches have led to opposing views. In some instances mitochondria have been shown to enter into secretory activity. In other cases it appears that they are unrelated to this cellular activity. The solution of the problem of the relationship of mitochondria to secretion has a significant bearing on the theory of Symbionticism, and will be discussed at greater length in the chapter on "The Behavior of Mitochondria."

In 1918 Portier published an extensive treatise entitled: "Les Symbiotes," in which he brought forth evidence to support his theory that mitochondria are bacterial organisms symbiotically combined with the cells of all higher organisms. Many of the views and the fundamental evidence that Portier submitted have not been acceptable to cytologists, bacteriologists, and biologists in general. He quoted an extensive literature which supports his views and we shall have occasion, in a later chapter, to draw upon these examples to illustrate the various degrees of microsymbiosis which may be found in nature. The crucial evidence submitted by Portier was unacceptable to bacteriologists in particular. This evidence had to do with the actual demonstration of the independent nature of mitochondria. Portier planted tissues in culture media and obtained bacterial growths in the media. An analysis of these experiments revealed possibilities of error which were not taken into

account by Portier. (The author published a critical analysis of Portier's experiments in 1923 (Wallin, '23).) The nature of the organisms cultured in Portier's experiments, apparently, had none of the properties ascribed to mitochondria; they not only resisted the action of acetic acid, alcohol and ether, but Portier maintained that the organisms retained life after being kept in absolute alcohol, for example, for very long periods. Such properties are incompatible with the known properties of living matter, or protoplasm. The Paris biologists who examined Portier's preparations were convinced that the growths obtained represented contaminations.

It is evident from this brief review of mitochondrial literature that misleading assumptions have served to color the interpretations in connection with mitochondrial research. The solution of the central problem in this field of investigation—the nature of mitochondria—gives promise of the most fundamental and far-reaching modifications in our conceptions of the cell. Certainly, the most generally accepted views on the nature of mitochondria have had no real basis for their pronouncement; nor have these views led the way to any real advance in a better appreciation of cell physiology.

CHAPTER III

The Bacterial Nature of Mitochondria

Although a great number of investigators have attributed diverse properties to mitochondria, the specific conception that mitochondria may be living bodies does not appear to have been seriously considered. On the contrary, this conception in Altmann's work was not accepted and again it was rejected in Portier's "Symbiotes." The outstanding morphologic characteristic of mitochondria is their resemblance to bacteria. While morphologic resemblance, considered by itself, does not necessarily imply relationship, it does, at least, suggest that possibility. The possible relationship between mitochondria and bacteria is further suggested by some of the properties exhibited by mitochondria, particularly their evident mode of multiplication by simple fission. So, also, the secretion activities that have been attributed to mitochondria by so many authors are indeed suggestive of the properties of living bodies.

When the literature up to the year 1918 was carefully examined, it became apparent that the characteristics attributed to mitochondria which differentiated them from bacteria were assumed rather than proved. The literature did not contain any reference to experiments that were intended to demonstrate specific differences in the two groups of bodies. It is evident that the necessity for experimental and specific determination of the relationship between these two groups of structures is essential before we can proceed intelligently to develop a logical conception of the nature of mitochondria.

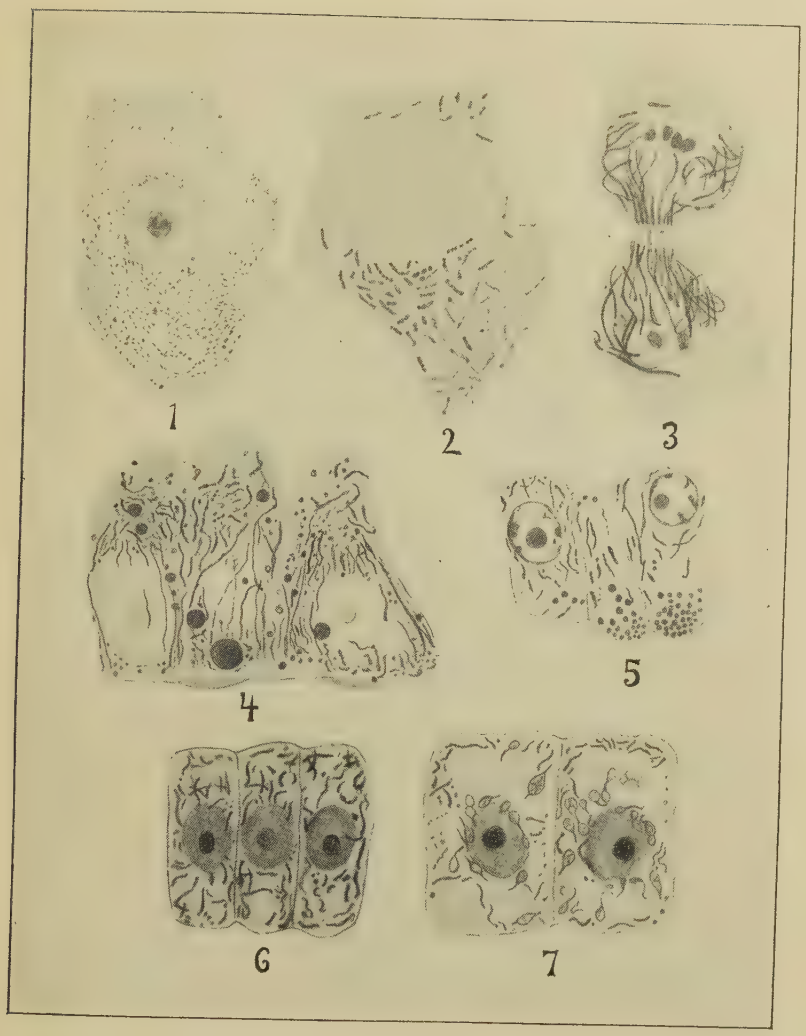
In the early part of 1919, I began a series of investigations on the nature of mitochondria. These investigations were

at first directed toward testing experimentally the reactions of bacteria to those agents that had been used in determining the "characteristics" of mitochondria. The complete details of these and the subsequent investigations may be found in the original papers, a list of which is given in the bibliography at the end of this book. The following resumé contains the chief results obtained in these researches.

The properties that had been specifically attributed to mitochondria and had been assumed to be distinct from those of bacteria are in the nature of reactions which will be considered under the following classifications: Staining properties, reactions to various chemical and physical agents, and thermal responses. The assumed chemical constitution of mitochondria was largely based on the sum total of these various reactions.

Staining properties. While most of the mitochondrial stains have not been considered specific for mitochondria, one stain, Janus green B (vital stain) has been placed in this category by Cowdry. A number of the mitochondrial stains were applied to bacteria and it was found that many strains of bacteria were stained like mitochondria by these methods. The Janus green B vital method was also applied to various strains of bacteria. Some varieties were very poorly colored by this method, but others, particularly the *pneumococcus* and *Bacterium coli* were stained in a homogeneous manner like mitochondria. It is evident from the results of these experiments that the staining reactions do not indicate any fundamental differences between mitochondria and bacteria.

Mitochondria were also subjected to bacteriological staining methods. This was done on smear preparations of tissues from fetal and young animals. They were treated like ordinary bacterial smears. The following bacteriological stains were used: Loeffler's methylene blue, pyronin-methyl green, the Giemsa stain and Gram's method. The mito-



MITOCHONDRIA OF VARIOUS CELLS

Fig. 1. Human spinal ganglion cell (after Cowdry). Fig. 2. Dog thyreoid cell (after Takagi). Fig. 3. Dividing insect cell showing mitochondrial division (after Bowen). Fig. 4. Excretory duct cells of testis of fish (after Duesberg). Fig. 5. Guinea-pig pancreas cells (after Bensley). Figs. 6 and 7. Cells from growing root-tip of barley. Starch grains forming in mitochondria in figure 7 (after Guilliermond).

chondria in the smears were distinctly stained by these methods. Gram's stain, however, did not bring them out as sharply as the other methods.

These two sets of staining experiments further indicate the danger of attributing specificity to staining reactions. These results do not belittle the value of various stains, but they indicate rather the limitations of staining reactions in determining the chemical constitution of small bodies that cannot be isolated for direct chemical analysis.

Chemical reactions. A number of investigators have observed that mitochondria are sensitive to certain chemicals. These chemicals are ether, alcohol, and acetic acid. Two of them are used extensively in histological technique, and this circumstance, perhaps, explains why mitochondria were not recognized at a much earlier date. It is usually stated that these chemicals dissolve mitochondria. This may not necessarily be the case, for the "dissolution" is usually determined by staining reactions. It is obvious that the reagent may alter these bodies so that they do not stain, and consequently they would not be readily detected in the cytoplasm.

The effects of alcohol, ether and acetic acid on bacteria have been determined. Various strains of bacteria were placed in shallow cups built up on microscopic slides, and the various solutions of acetic acid and alcohol were then introduced. Ether was used in concentrated form. The chemicals were allowed to remain for a time and then evaporated, leaving all solid matter on the slide. After floating a thin layer of celloidin over the slide and permitting it to harden, the preparations were stained. Microscopic examination of these preparations showed a variety of reactions of the various strains of bacteria in the different solutions. In some cases, the organisms remained intact, but were unstained. In some instances, an amorphous mass is all that remained on the slide; it was stained in some specimens,

but not in others. In other cases the organisms were well preserved and deeply stained.

These experiments failed to demonstrate any significant differences in the reactions of bacteria and mitochondria to ether, alcohol and acetic acid.

Reactions to physical agents. Mitochondria are known to be sensitive to mechanical manipulation, desiccation and slight variations in the physical environment. They fragment readily. This sensitiveness of mitochondria is usually spoken of as fragility. It has been assumed that bacteria do not possess such a fragility and that this characteristic differentiates them from bacteria. Guilliermond ('19), in particular, has stated that there are no bacteria known that are as fragile as mitochondria.

Experiments were devised in which the tissues of fetal, young and old animals were smeared on glass slides, the film dried, fixed and stained by various mitochondrial and bacteriological methods. It was at once apparent in these preparations that the fragility of mitochondria varies in the different tissues, *and with the age of the animals.* In the smears from adult animals mitochondria were seldom visible either in the intact cells in the preparation or in the cytoplasm of the ruptured cells. In the tissue smears from young animals, the mitochondria were present in the intact cells, but seldom in the cytoplasm of ruptured cells. The smears from fetal tissues invariably contained mitochondria in great numbers in the fragments of ruptured cells as well as in those still intact.

These experiments seem to indicate that fragility of mitochondria increases directly with the age of the animal.

Fragility in bacteria was not investigated experimentally. Bacteriological literature contains ample evidence that bacteria vary considerably in fragility. One microörganism, in particular, exhibits a degree of fragility properties that, apparently is as great as that exhibited by mito-

chondria. This organism (there is some question in regard to its true bacterial nature), which is associated with the disease Rocky Mountain spotted fever, has been investigated recently by Wolbach who has taken cognizance of its fragile nature. A number of related forms, spoken of collectively as "Rickettsia bodies," also, are characterized by their fragile nature.

Fragility is thus not a specific characteristic of mitochondria, but in known microörganisms it appears to be a property acquired in the development of a symbiotic existence.

Thermal responses. A number of investigators have found that mitochondria are apparently destroyed when the tissues containing them are exposed to a temperature of 48° to 50°C. for a comparatively short period. It is well known that microörganisms exhibit a wide range of reactions to temperature. Some organisms live constantly in hot springs where the temperature is not far below the boiling point; others thrive in cold regions where the temperature may be far below zero.

Experiments were made in which some ten strains of bacteria were placed in vials containing physiological salt solution and incubated at a temperature of 49°C. for half an hour. The organisms were examined before incubation to determine if the physiological salt solution affected their morphology. They all appeared to be intact. After the incubation, a drop from the bottom of the vial was removed with a pipette, smeared on a slide and allowed to dry. A celloidin film was then floated over the dried smear to fix it on the slide. In these experiments it was found that some of the strains of bacteria were apparently unaffected. Other strains, however, had "dissolved" or disappeared; amorphous masses, stained in some cases, were all that remained of the organisms.

These experiments failed to demonstrate any fundamental

difference in the reactions of bacteria and mitochondria to temperature.

Chemical constitution. The chemical nature of mitochondria has been presumed to be a phosphatid combined with albumin. The reasons for this assumption were discussed in the previous chapter. While the evidence, so far as it goes, points to the presence of phosphatids in the mitochondria of the liver cells, it is probable that they are also present in other mitochondria. There is no evidence, however, to indicate that the phosphatid-albumin complex is the *only* constituent of mitochondria.

Certainly the chemical determinations that have been made on mitochondria and bacteria are not sufficient to justify the conclusion that the two groups of structures are fundamentally different in chemical constitution. So far as the reactions of mitochondria to physical, chemical, and thermal agents have been the basis for determining their nature, the responses of bacteria to these same agents would rather suggest a similarity of the two groups of structures.

In a series of studies on ferment action, Joblin and Petersen ('14a, b, c) have determined the presence of lipoids in bacteria. It may not be amiss to quote their opinion in this connection. "Bacteria do, however, contain fats and lipoids in varying amounts, which because of their marked effect on surface tension, would for purely physical reasons tend to become concentrated at the periphery of a colloidal system such as the bacterial protoplasm. With or without a morphologically distinct limiting membrane we can reasonably assume that the external surface of the bacterial cell is potentially lipoidal."

The results of these experiments and the analysis of mitochondrial literature failed to reveal any evidence that excluded the possibility that mitochondria may be bacterial in nature. On the other hand, many properties that have

been attributed to mitochondria seem to find their most logical explanation in a conception of their bacterial nature. Obviously, it became necessary to attempt experimental and specific determination of the nature of these bodies. It is evident that, if mitochondria could be induced to grow independently of the cell, there could be no question regarding their bacterial nature.

The problem at the beginning was beset with a number of difficulties. Bacteriological literature contained a considerable number of references to experiments on growing bacteria in culture media planted with tissues from presumably healthy, normal animals. These growths had been thought to arise from extra-cellular, non-pathogenic, symbiotic microörganisms. There was nothing to indicate that they had not developed from the mitochondria normally present in the cells of the explanted tissues. It was also presumed that these symbiotic microörganisms were acquired by the animal after birth. Hence, to avoid this uncertainty, it was deemed advisable to choose for experimentation those tissues in which the possibility of contamination would be reduced to the minimum.

The tissues of the fetus and the new-born, at least theoretically, should be uncontaminated by extraneous microorganisms and consequently were chosen for the proposed culture experiments.

The problem of a proper culture medium offered, perhaps, the greatest difficulty. It appeared that it would be necessary to use a medium which possessed the essential attributes of cytoplasm, inasmuch as mitochondria normally live in a cytoplasmic environment. This idea suggested the use of some kind of living protoplasm as a culture medium, but on further reflection it was obvious that any positive growth in such a medium, would not furnish conclusive evidence on the point at issue. At best, it would permit of various interpretations which would only lead back

again to the original status of the problem. It was decided to test out various bacteriological culture media and to devise new media.

Experiments were then made in which liver tissue from fetal and new-born rabbits was planted in a number of the more commonly used culture media. The results of these experiments were chiefly negative. A human blood medium was tested, resulting in a positive bacterial growth. The organisms which developed as a surface growth had some unusual characteristics. It was difficult to procure sufficient quantities of human blood to make this medium on a large scale, and satisfactory sterilization of the medium was extremely difficult. Special media were then devised on the principle of the meat-infusion medium, in which rabbit liver replaced the meat. The media were adjusted to various hydrogen-ion concentrations, ranging from pH 6.4 to pH 8.3. When bits of liver tissue from fetal and new-born rabbits were planted in these media, surface organisms developed in a number of the culture tubes. In practically all of the tubes that did not contain the surface organisms, a peculiar clouding developed in the culture medium about the planted liver tissue. When a part of this clouded medium was removed from the tube, smeared on a slide, dried, fixed, stained and examined microscopically, it did not contain any visible bodies that had the appearance of bacteria. When wet preparations of the clouded material were examined microscopically, small coccoid bodies could be seen. The clouded culture media and planted tissues were removed from a number of culture tubes, fixed in a mitochondrial fixative, embedded in celloidin, sectioned and stained. Microscopic examination of these preparations revealed myriads of coccoid bodies in the clouded medium. It was evident from the results of these experiments that the coccoid bodies of the clouded medium were fragile, and disappeared in the smear preparations from the clouded medium.

In all these culture experiments the possibility of contamination was constantly kept in mind and guarded against. All instruments and vessels that were used were thoroughly sterilized. The fetus or new-born rabbit, after decapitation was saturated with 95 per cent alcohol. The instruments employed in opening the abdomen and removing the liver were always sterilized in the flame immediately before use. The liver was quickly removed to a sterile petri dish, cut into pieces by inserting a sterile scalpel under the lid. Pieces of the liver tissue were then planted in the media by the usual bacteriological technic.

The "clouded growths" obtained in these experiments appeared to be the more significant, since they developed consistently. It appeared possible, however, that these coccoid bodies of the clouded medium might be the result of some precipitation of a colloidal nature induced by the planted liver tissue. This possibility was further suggested by the failure of all attempts to subculture them. However, the clouded medium was examined with the dark field microscope in the hope of determining if the coccoid bodies exhibited true movement. In a number of such examinations true movement was observed. In some instances, the minute bodies were seen to move across the entire microscopic field. These movements were not confused with Brownian movement, which was abundantly present in the preparations. These observations strengthened the idea that the coccoid bodies might be living organisms.

The experiments completed up to this time, while not conclusive, certainly enhanced the probability of demonstrating the bacterial nature of mitchondria. An analysis of the nature and extent of growth, particularly the deep clouded growth, appeared to indicate that the media were deficient in some essential food material. It was suggested by Dr. A. I. Kendall[1] that possibly the (limited)

[1] Personal communication.

growth observed was dependent upon food materials present in the planted liver tissue. When this material is exhausted, growth necessarily ceases. The pertinence of this suggestion was strengthened by the observation that the extent of cloudiness about the planted tissue bore a direct relationship to the size of the tissue fragment explanted. The problem then resolved itself into discovering what food material might be deficient in the culture media. It appeared likely that it was some nitrogenous foodstuff. Various sugars and salts had been tested with practically no indication of variations in the results. Nitrogenous food had been supplied to the media from two sources: the infused liver and peptone; but it appeared probable that the nitrogen from these sources was not in a form available for mitochondrial utilization. This naturally led to an analysis of the possible sources of nitrogen for mitochondria in the living cell. Obviously, nitrogen compounds in the living cell have two sources of origin: those brought to the cell in the form of amino acids by the blood, and the waste products of nitrogenous metabolism, chiefly in the form of urea. On the basis of these speculations, a new series of culture media was devised in which various amino acids and urea were used. These chemicals were added to the rabbit liver infusion medium in varying dilutions.

The preliminary experiments consisted in testing the various amino-acid and urea media. Of the amino acids, only tryptophane appeared to satisfy the conditions necessary for free surface growth of mitochondria. It is possible that certain combinations of other amino acids might have supported growth; a few were tested with negative results. The urea medium also satisfied the conditions for independent mitochondrial growth. In general, it appeared that growth was more luxurient in the urea media than in that containing tryptophane.

Attention was again turned to the deep clouded medium

of the earlier experiments. A number of specimens were subcultured into the new media. In a large number of the subculture experiments, the coccoid bodies of the clouded media developed definite surface growths. Deep clouded growths that were several weeks old did not develop into surface growths so readily as did younger cultures. These results in subculturing the deep clouded growths removed the last vestige of doubt concerning the bacterial nature of the coccoid bodies.

There is no occasion to confuse the coccoid bodies observed in these experiments with the "artificial bacteria" produced by Fischer ('99), Löwschin ('13) and others. Precipitation of colloids may be produced in solutions by various methods, the precipitate assuming various forms and characteristics. Some of these may have a morphological resemblance to the coccoid bodies and to bacteria. Evidence is lacking, however, that the precipitates can be subcultured upon the surface of solid media and caused to multiply, properties shown to be possessed by the coccoid bodies and known to be characteristic of most bacteria.

A large number of culture experiments were then made, in which liver tissues from fetal, new-born and adult animals were planted in the tryptophane and urea media. When tissues from the fetus and new-born were used, surface growths developed in practically all cases with either media. In a few experiments in which adult tissue was used surface growth appeared in nearly all of the tryptophane media tubes, but in none of those containing urea.

The morphologic nature of the organisms that developed in the culture experiments was puzzling. The constancy with which surface growths appeared, and the precautions used in the experiments appeared to rule out contamination, nevertheless, the morphology of the organisms seemed to point to it. The mitochondria of the liver cells are mostly globular bodies. A few ovoid forms are usually seen in the

cell, and occasionally a distinct rod-shaped body may be observed. While many of the surface growths in the culture experiments were pure cultures of cocci, the majority, perhaps, consisted of diplococci, clumped or agglutinated cocci, and a type of coccus that I have called "ring-forms." In a few instances, rod-forms predominated in the surface growths. Subcultures of these organisms furnished a clue to the interpretation of this variation in morphology. In some instances, where the initial growth consisted of a pure culture of cocci, the subcultures were found to contain all the varieties of forms mentioned above (see plate III, facing p. 51). An extensive study of this pleomorphism was made, and it became evident that slight variations in the culture medium were sufficient to bring about remarkable variations in the morphology of the microörganisms. These experiments will be considered more fully in the next chapter.

It was obvious that the results obtained so far did not constitute conclusive evidence that mitochondria are bacterial in nature. There was, after all, no direct evidence that the organisms grown in the culture media had originated from the liver cells. It was also possible that the tissues used for planting might contain congenital contaminations or infections. This possibility did not appear likely, for, when the normal unincubated control tissue (a part of the same liver used for planting) was minutely examined there was no indication of any organism other than the mitochondria. It again became necessary to seek for some method that would conclusively demonstrate the source of the variable organisms in the cultures.

A new set of experiments was performed in which liver tissue was planted in the same kind of media previously used, but the tissue was permitted to incubate for only a short time (from two to twenty-four hours in the different tubes), when it was removed and treated with a mito-

chondrial fixative. When these incubated tissues were examined microscopically, it was found that the mitochondria had *changed in morphology within the cell.* The pleomorphism here observed was identical with that noted in the case of mitochondria grown on the surface of the media in the previous experiments. Figures 1 to 12 on plate II are camera lucida drawings of some of the organisms that developed on the surface of the cultures. Figure 13 is a camera lucida drawing of a piece of normal unincubated liver tissue from a rabbit showing the normal appearance of the mitochondria. Figures 14 to 18 are similar drawings of liver tissue from the same rabbit as shown in figure 13, after being incubated in urea-liver media at 37.5°C. for two, four, six, eight and six hours, respectively. It is evident, after an examination of these figures, that the mitochondria changed in morphology when the tissue was incubated. It is also evident that the organisms that had developed on the surface of the media are identical in morphology with the pleomorphic mitochondria within the incubated liver cells.

The vagaries of contamination in bacterial work are a veritable "bug-a-boo" to the investigator. The bacteriologist has learned that error from contamination often leads him astray in the search for truth. Sometimes he traces the source of his contamination to the glassware used, and which he had sterilized in the usual manner. In other cases, the contamination, apparently, crept into the media during the manipulations of planting. It seems to me, that error from contamination is ruled out in this last set of experiments. I have attempted to find some explanation other than a mitochondrial origin for these organisms. The tissues during explantation might possibly become contaminated, but it is impossible to conceive of such contaminating organisms penetrating to the interior of cells in the deeper part of the tissue and multiplying to

such numbers in so short a time. Again the constancy of the organisms in different experiments is against the probability of contamination from the air. *These facts, apparently, admit of no other interpretation than that mitochondria are living organisms, symbiotically combined with the cells of plants and animals.*

PLATE II

All illustrations are camera lucida drawings made at the same magnification.

FIG. 1. Organisms developed on surface of culture medium when new-born rabbit liver was planted in a rabbit liver medium (D), pH 6.6.

FIG. 2. Organisms grown from new-born rabbit liver in rabbit liver medium (D), pH 7.4.

FIG. 3. Organisms grown from new-born rabbit liver in rabbit liver medium (D), pH 8.2.

Fig. 4. Organisms grown from new-born rabbit liver in rabbit liver urea medium (D), pH 7.4.

FIG. 5. Subculture of organisms of figure 2 into a different rabbit liver medium (C), pH 6.0.

FIG. 6. Organisms grown from new-born rabbit fat in nutrient agar tryptophane medium, pH 7.5.

FIG. 7. Subculture of clouded medium into rabbit liver urea medium (D), pH 8.2.

FIG. 8. Subculture of clouded medium into nutrient agar tryptophane medium, pH 7.5.

FIG. 9. Subculture of clouded medium into nutrient agar urea medium, pH 6.5.

FIG. 10. Subculture of clouded medium into rabbit liver medium (F), pH 7.5.

FIG. 11. Subculture of an upgrowth into nutrient agar, pH 6.5.

FIG. 12. Subculture of an upgrowth into rabbit liver medium (F), pH 7.5.

FIG. 13. Normal liver of a three-week-old rabbit (K*).

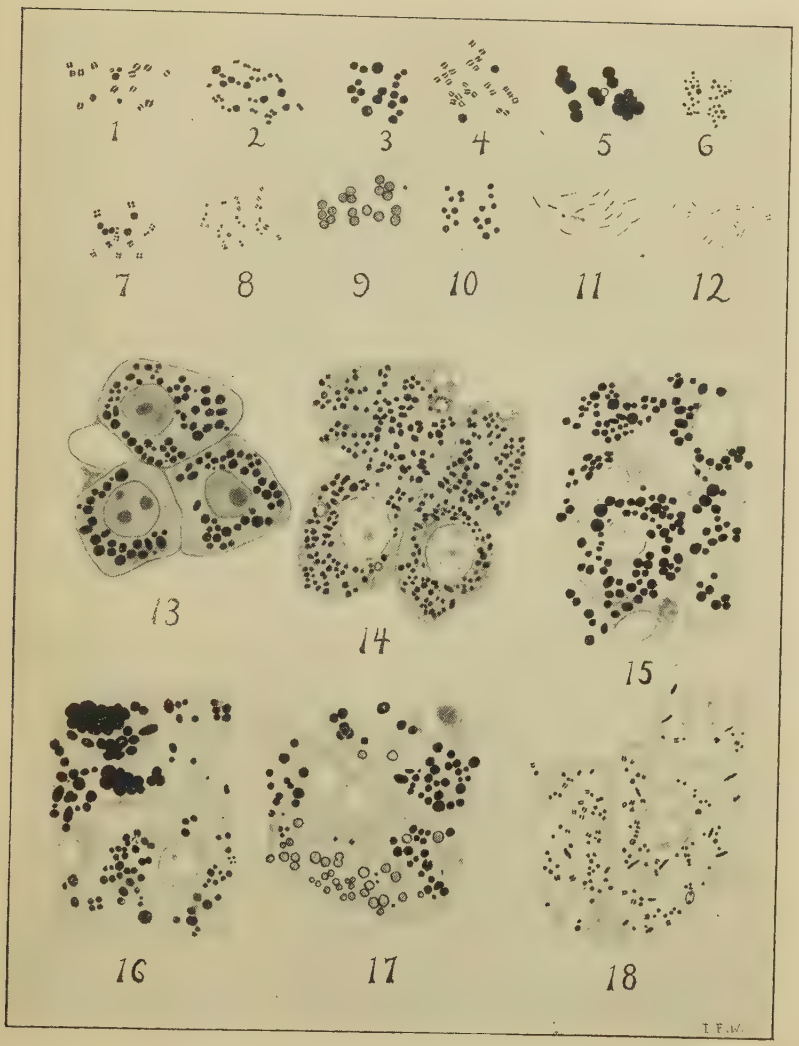
FIG. 14. Liver of rabbit K* after 2 hours incubation in rabbit liver urea medium (F), pH 7.5.

FIG. 15. Liver of rabbit K* after 4 hours incubation in rabbit liver urea medium (F), pH 7.5.

FIG. 16. Liver of rabbit K* after 6 hours incubation in rabbit liver urea medium (F), pH 7.5.

FIG. 17. Liver of rabbit K* after 8 hours incubation in rabbit liver urea medium (F), pH 7.5.

FIG. 18. Liver of rabbit Sc. after 6 hours incubation in rabbit liver urea medium (F), pH 7.5.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The major portion of these culture experiments have been made with liver tissue of fetal and new-born rabbits. Other tissues of fetal and new-born rabbits, guinea pigs, dogs, and cats have also been tested. Growths similar to those described have been obtained from fat, kidney and suprarenal of the above named animals. There is no reason to believe that the mitochondria of the liver are fundamentally different from the mitochondria of other cells, and that they alone are bacterial in nature.

The question has been raised, and it deserves consideration here: What grounds are there for calling mitochondria "bacterial in nature" or bacteria; why are they not to be considered merely a part of the cytoplasm which has properties of self-perpetuation and independence under proper artificial conditions? This is a pertinent question, and it strikes at the root of the theory that I have advanced in explanation of the fundamental significance of mitochondria. The complete answer to the question can not be given in a few words, for in the attempt to answer this question one must deal with indefinable matter. In the first place, if we seek a definition of bacteria, we are unable to find an adequate one in the text-books on bacteriology. Indeed, we have a mental conception of a type of organism when we use the word "bacterium." Morphologically, we differentiate bacteria from unicellular plants and animals by the absence of an organized nucleus. This does not constitute a definition of bacteria, however, nor would a catalogue of their physiological and morphological attributes constitute such a definition. A definition would have to go farther and include a statement of *what they are in relation to the earth and the life that it contains.* There has been, in recent years, a growing conception of what bacteria really are. The suggestion that they are *primordial* organisms indicates such a trend. Again, the difficulty of distinguishing between some of the lowly, supposedly, pro-

tozoan microörganisms and the bacteria, led Haeckel to propose the term "protista" to include a lowest order of living organisms, a *distinct kingdom* of living things. In this connection too, the suggestion has been made that the "bacteria" are the primordial organisms from which all higher forms of life have sprung. (So far as I know, no one has ever attempted to expound a concrete theory of the nature of this early evolution. The evidence which will be presented in a later chapter of this book, establishes a basis for a better understanding of the nature of such an evolutionary process.)

The second factor that complicates the question of the evidence or basis for calling mitochondria bacteria, concerns our knowledge of protoplasm and particularly cytoplasm. The nucleus has long been looked upon as the controlling factor in the cell. The center of vital activity is supposedly located in the nuclear portion. Bits of cytoplasm removed from the cell do not continue to grow and function as an independent entity. The remarkable analyses of chromosomes in relation to heredity, that have recently been made by students of genetics would appear to exclude any possible independent participation of cytoplasmic bodies. On the other hand, Conklin and Wilson believe that the cytoplasm participates in hereditary transmission. It might be argued that parts of the cytoplasm should be capable of independent existence. Such arguments might proceed along various lines. It might be assumed that the cytoplasm as such (not the formed bodies that it contains) may have such properties of self perpetuation. Or it might be assumed that formed bodies of the cell having these properties are a part of the cytoplasm. This raises a subtle question which must be left for the future to determine. Mitochondria, apparently, represent the end result of the development of an absolute microsymbiosis. What is the nature of the intimate relationship

of mitochondria to the cell? Do the mitochondria become a part of the cell cytoplasm, or do they retain their individuality? The fact that they may be induced to exist independently of the cell would appear to indicate that they do retain their individuality, but this does not necessarily indicate an absolute independence. Again, the observations of Chambers in which he apparently saw mitochondria disappear by mixing with the cytoplasm, has a bearing on the question. It will require special investigations to determine the intimate physical relationship of mitochondria to the cytoplasm. The possible reactions of microsymbionts in the cytoplasm of the host cell is discussed in later chapters.

The evidence for calling mitochondria bacteria, rests upon the following attributes: Their general behaviour in the cell is similar to that of known microörganisms which live symbiotically in the cells of higher organisms; for example, the root-nodule bacteria of legumes. When grown independently in artificial culture media, they behave in all observed particulars like bacteria. They divide like bacteria. They are similar to bacteria in structure and shape. They exhibit no cultural characteristics foreign to bacteria.

CHAPTER IV

The Behavior of Mitochondria

One of the more characteristic attributes of mitochondria that may be gathered from a perusal of the literature is their property of changing form or pleomorphism. As early as 1897, Benda showed that the mitochondria in spermatogenesis transform into the spiral filament of the spermatozoon. Later investigators have traced the "transformation" or differentiation of mitochondria of various tissue cells into a large variety of end-products (myofibrils, connective tissue fibrils, secretion droplets, prosecretions, pigment granules, plastids, etc.). Many of these so-called transformations have been questioned on the basis of an assumed phospholipin-albumin constitution of mitochondria, which also presupposed a "passive" nature of these bodies.

The relationship of mitochondria to secretory processes appears to have partially weathered the storm of criticism, and is still accepted by a large number of investigators. Altmann was the first to find that the granules (mitochondria) are related to secretory processes in the wax glands of the ear and the submaxillary gland. Regaud and Mawas ('09) confirmed Altmann's work on these glands, and further showed that the granular or coccoid mitochondria arise from the bacillary or filamentous forms by a process of fragmentation. O. Schultze ('11) investigated the problem and concluded that the secretory granules originate from mitochondria. Debeyre ('12) investigated the submaxillary gland of the rabbit, and with the aid of vital stains (Janus green and neutral red) he was able to observe the transformation stages of mitochondria into secretion granules. Guieysse-Pelissier ('11) examined the

submaxillary gland of the mouse, and was of the opinion that the secretory granules originate from the mitochondria. Levi ('12), on the other hand, advanced the idea that mitochondria are permanent cell-organs, and while they may be involved in the secretory process they do not transform as such into secretion granules.

In a series of papers, Moulon ('10a, '10b) has described the transformation of mitochondria into secretion in some of the cells of the suprarenal gland. The rod-shaped mitochondria with blebs become globular, later agglutinated masses, and finally the entire cell is filled with the mitochondrial product which he calls a "prosecretion." Takagi ('22) described a similar chain of events of mitochondria in the thyroid gland of the dog. Ma ('25) concluded that mitochondria are concerned in thyroid secretion in the albino rat. Key ('25), however, does not believe that mitochondria are responsible for the specific activity of the cells that contain them.

Cowdry's general discussion of the relationship of mitochondria to secretion may be considered as reflecting the opinion of those who are against the idea that mitochondria are concerned in secretion. He says: "I incline strongly toward Regaud's eclectosome theory (1919a, p. 919), according to which mitochondria play the part of plasts choosing and selecting substances from the surrounding cytoplasm, condensing them and transforming them in their interior into infinitely diverse products; but I would venture to emphasize the fact that in all this the mitochondria may be acting in an entirely passive manner as a vehicle, taking up materials by virtue of their phospholipin constitution, or on account of physical forces acting on their surfaces, or for other reasons, and that the optically homogeneous ground-substance of the cytoplasm may be the active and essential agent in this as in so many other vital manifestations." It is difficult to understand why

one should assume that mitochondria are "passive" bodies, if one accepts the statement that they have selective and secretory properties. These properties of selecting substances from the environment and elaborating "infinitely diverse products" is one of the outstanding characteristics of bacteria and all living matter.

In discussing the mechanism of secretion it becomes necessary to consider briefly, ideas recently advanced on the functional significance of the Golgi apparatus. This cell structure was first demonstrated in gland cells by Negri ('99). Numerous investigations have been conducted upon the Golgi apparatus, but until recently no acceptable idea as to its function has been advanced. Nassonov ('23) after painstaking investigations arrived at significant conclusions regarding its activities. He believes that secretion is intimately connected with the Golgi apparatus and has offered various suggestions regarding the probable nature of the mechanism through which it acts. More recently Bowen ('24) has presented other evidence in support of Nassonov's conclusions. Bowen quotes opposing views of investigators as to the rôle played by mitochondria in secretion, and states: "In view therefore of the conflicting statements of fact and the known sources of error, it appears probable that the origin of secretory granules from mitochondria, ergastoplasm, and related cytoplasmic elements must be looked upon as unproved and of doubtful utility as a working hypothesis."

While space will not permit an extensive discussion of these apparently conflicting views on secretion, it is pertinent to direct attention to other investigations which appear to offer a solution of the difficulties. Two papers in particular were published in 1925 which are significant in this connection. Hirschler ('25) emphasizes the fact that mitochondria and the Golgi apparatus are similar in staining reactions. Karpova ('25) concludes from observations on

living as well as fixed material, that there is nothing to indicate a fundamental difference between the two structures. While she admits that she has not observed mitochondria transforming into dictosomes (Golgi bodies), nor does the literature refer to any such observations, she maintains that the apparent identity in chemical nature points to a similarity in origin.

It would be premature to draw definite conclusions as to the relationship of these two groups of cell structures on the evidence at hand. However, it is proper to suggest the probability that mitochondria and the Golgi bodies are at least related structures, and that in some instances secretion may be derived directly from the mitochondria, while in other cases the mitochondrial bodies first transform into a reticulated apparatus before secretory activities begin. Evidence has been presented by the author (Wallin, '24) that the mitochondria in a single cell are not all alike. When using the Giemsa stain, some mitochondria stain blue while others are stained red. Karpova emphasizes the fact that there is a greater difference between the mitochondria and the chloroplasts derived from them than there is between mitochondria and dictosomes. In this connection it should be further emphasized that there is no good reason for assuming that the secretory mechanism is the same in all kinds of cells. On the contrary, it is well known that there are gross variations in the behavior of the cells of different secreting glands. Does this not at least allow for variation also in the internal secretory mechanism.

Ludford ('25a) discusses the rôle of mitochondria and the Golgi apparatus during secretion in the epididymis, and says: "At the onset of secretion the mitochondria increase in numbers, and there is a diminution after secretory activity, so that they contribute to the production of formed bodies seems practically certain, but the exact nature of their contribution is a matter for theoretical discussion

rather than practical demonstration." In another investigation on the sebaceous gland cells, Ludford ('25b) says: "Mitochoncria do not appear to be directly concerned in the formation of the secretion."

From an analysis of the literature on secretion, it is evident that the internal mechanism of secretion varies in different glands and in different animals. Investigations have demonstrated that in some glands the mitochondria are concerned in secretion. The recent experimental investigations of Takagi ('25) in which he used various means to stimulate secretory activity in the submaxillary gland of the cat demonstrate conclusively that mitochondria play a rôle in submaxillary secretion. Cramer and Ludford ('26) demonstrated a relationship of mitochondria to the functional state of the thyroid gland. Horning ('26) found mitochondria in the food vacuoles of paramoecium, indicating a digestive function. The manner in which mitochondria enter into secretory activity appears to vary in different kinds of cells. In some cases the mitochondria appear to transform directly into the secretion; in other instances the secretion appears to be related to the mitochondria, but not in the nature of a transformation. In still other cases the Golgi apparatus appears to be directly concerned in the secretory process. The question that presses for solution in connection with the mechanism of secretion is: What is the relationship between mitochondria and the Golgi bodies?

While some investigators maintain that the Golgi bodies are chemically related to mitochondria, others hold that they are distinct and of a different nature. The basis for these opinions rests on the staining reactions of the two structures. It is pertinent to emphasize the fallacy of attributing specificity to staining reactions. It is conceded that slight variations in staining reactions can be detected between mitochondria and the Golgi apparatus; but what

significance should be attached to these slight variations? It has been shown that mitochondria vary in their staining reactions. So far as the researches on these two groups of structures have been carried, there is no conclusive evidence to show that mitochondria and the Golgi bodies may not be related structures. When one contemplates the modifications that mitochondria undergo in the formation of the nebenkern, a transformation of mitochondria into a net-like structure, or a laminated structure, appears to be a simple process.

Other variations in the behavior of mitochondria have been recorded in the literature. It has been observed by a number of investigators, and I believe it is generally accepted, that the amount of mitochondria varies with the age of the cell. Young growing cells contain a larger number of mitochondria than those that are old and senile. It has also been observed by Romeis ('13), Torraca ('14a, '14b), and others that the mitochondria increase in numbers in regenerating tissues. Of particular interest are the modifications attributed to mitochondria in abnormal or pathological tissues. The researches in this field have been fairly numerous, but the results have not led to any particular fundamental conception of the nature of mitochondria.

Observations on the mitochondria in pathological tissues have indicated, in some cases, an alteration in the number of mitochondria, and in other instances, pleomorphic modifications have been mentioned. The observation of Goetsch ('16), are particularly significant, in as much as he correlates the number of mitochondria present in the thyroid with various diseased conditions of the gland. Goetsch found a profound reduction of mitochondria in colloid goiter. In cases of adenoma with hyperthyroidism and exophthalmic goiter he found an increased number of mitochondria. Quite recently, Seecof ('25), has found definite mitochondrial changes in the thyroid running parallel with experimental

hyperplasia and hypoplasia of the gland. Other pathological tissues have been investigated by various authors, but the results, apparently, have not been so definite.

Experimental modifications of mitochondria have been induced by various methods. Ciaccio in 1913 observed the changes in the mitochondria of various tissues after phosphorus poisoning. The following year Mayer, Rathery and Schaeffer investigated the effects of phosphorus poisoning on the mitochondria of the liver. They found pleomorphic modifications of the mitochondria as well as destruction of these bodies. In 1916, W. J. M. Scott investigated the effects of experimental phosphorous poisoning on the mitochondria of the pancreas in white mice. Scott's results are particularly interesting when compared with the behavior of mitochondria in culture media. He found that the first change observable in the mitochondria in mild poisoning is a loss of the bleb-like swellings normally present in pancreas mitochondria and a thickening and shortening of these bodies. When the poisoning is carried further, the mitochondria clump together like agglutinated bacilli. In more severe cases the agglutinated forms appear to fuse and completely lose their identity. In some of the stages described by Scott, he found globular bodies which appeared to contain a vacuole. In some instances, only a peripheral ring stained with the dye used, the center appearing clear. These forms, apparently, are identical with the pleomorphic mitochondria, to which I have previously referred as "ring forms."

Nicholson, in 1924, used various means to modify experimentally the mitochondria of the thyroid. The blood supply to the gland was interfered with in one set of experiments. After three days the mitochondria which, normally, are filamentous were shorter and more granular. After the tenth day they had become spherules.

In another set of experiments, about three-fourths of the

gland was extirpated, and the effects on the mitochondria noted in the remaining portion of the gland which presumably was called upon to increase its activity. The mitochondria were examined at various periods after the operations. It was found that they had not altered in morphology, but had increased considerably in numbers. Nicholson also tested the effects of various gases and chemicals. In some instances the mitochondria exhibited pleomorphism.

The investigations of Bowen ('19–'22) and others, on the behavior of mitochondria in spermatogenesis have brought to light some interesting activities of the mitochondria of germ cells. It has been shown that the mitochondria in the male germ cells of certain insects come together and fuse into a single body, the nebenkern. This body is not homogeneous in structure, but is composed of at least two distinct materials which Bowen was able to distinguish by means of staining reactions. In later spermatogenetic activity, the nebenkern becomes peculiarly laminated.

These activities, that have been so clearly described and illustrated by Bowen, appear, on first analysis, to be quite unrelated to any comparable process in the bacteria. Löhnis, in his monograph on the "Life Cycles of Bacteria," however, has described and illustrated processes that are identical with the early changes described by Bowen. According to the conclusions of Löhnis which are based not only upon his own observations, but also correlated with those of many other investigators, bacteria possess a life cycle. One step in the process consists of the fusion of a number of bacteria into an amorphous mass which Löhnis has named the "symplasm." Later, individual bacteria become separated from this mass or symplasm, and again become independent organisms. While many bacteriologists have not accepted this work of Löhnis, I know of no definite evidence against it.

J. Terrill Scott ('26), working in this laboratory, has studied the responses of mitochondria to variations in the hydrogen-ion concentration. When liver tissues were incubated in physiological saline solutions of different pH values for short periods of time, there was a variation in the behavior of the mitochondria. He also found that the staining reaction of the incubated mitochondria varied under these conditions. Jessen ('25), produced degeneration of mitochondria in kidney cells by the injection of bacteria, diphtheria toxin and distilled water.

In the experiments described in the preceding chapter, it was noted that the cultured mitochondria did not always have the same morphology as those in the normal liver tissue. This peculiarity was noticed in the first surface growths that were obtained. A large series of subculture experiments were made from the initial growths in order to study more minutely the pleomorphic possibilities of the cultured mitochondria.

The first subculture experiments were made with a coccus which developed in a rabbit liver medium eight days after it had been planted with liver from a new-born rabbit. When the organisms were subcultured into beef heart media of various hydrogen-ion concentrations from pH 7.1 to pH 7.5, the growths in these media varied in size according to the pH value of the medium. It was possible to transplant any of these subcultured organisms into a medium with a particular pH value and obtain organisms of a uniform size. After these cultures had been kept a little more than a year in cold storage, they were found to have lost their specific response to the hydrogen-ion concentration of the medium. The size would vary in media of different pH values, but the variations were erratic in character.

A large number of subcultures were made from the initial growths that developed in the urea and tryptophane media. The highly specific response obtained in the earlier subcul-

Feb. 28, 1924
0'-21. Rabbit fat planted in nut. agar. pH 7.5 — +
Subcultures March 11,1924
March 12,'24
April 1,'24
0'- 1. 0'-21 into rabbit liver 2 pH 5.2 +
0'- 3. " " " " " pH 5.6 —
0'- 4. " " " " " pH 5.8 +
0'- 5. " " " " " pH 6.0 +
0'- 6. " " " " " pH 6.2 +
0'- 7. " " " " " pH 6.4 +
0'- 8. " " " " " pH 6.8 +
0'- 9. " " " " " pH 7.0 +
0'-10. " " " " " pH 7.2 +
0'-11. " " " " " pH 7.6 +
0'-12. " " " " " pH 7.8 +
0'-13. " " " " " pH 8.0 +
0'-14. " " " " " pH 8.4 +
0'-15. " " " " " pH 8.6 +
0'-16. " " " " " pH 8.8 +
0'-17. " " " " " pH 9.0 +
0'-18. " " " " urea pH 7.0 +
0'-19. " " " " tryp. pH 7.0 +
March 12, 1924
March 13,'24
April 1,'24
0'-20. 0'-21 into nutrient agar pH 6.5 +
0'-21. " " " " pH 7.0 —
0'-22. " " " " pH 7.5 +
March 13, 1924
March 14,'24
April 1,'24
0'-23 0'-5 into nutrient agar pH 6.5 +
0'-24 " " " " pH 7.0 —
0'-25 " " " " pH 7.5 +
0'-26 0'-19 " " " pH 6.5 +
0'-27 " " " " pH 7.0 +
0'-28 " " " " pH 7.5 +
0'-29 " " " " urea pH 7.5 +
0'-30 " " " " tryp. pH 7.5 +

PROTOCOL OF SUBCULTURE EXPERIMENT

Original upgrowth (0'-21) developed after four days incubation. Character of organisms shown by means of camera lucida drawings at right.

ture experiments, however, were not again observed, that is, the production of a definite size of organism in a given hydrogen-ion concentration of the medium. The results that were obtained in the later subculture experiments, however, appear to be more significant in relation to the known behavior of mitochondria as indicated by the experiments of the investigators quoted above.

Plate III is a reproduction of the protocol of a subculture experiment. The character of the organisms that developed in each subculture is shown by means of the camera lucida drawing at the right. The initial growth from which these subcultures were made consisted of a pure culture of cocci, represented in the upper part of the plate. The original smear of the initial growth was carefully searched, but the only type of organisms that could be seen were single cocci. A study of this protocol reveals the peculiar and delicate pleomorphic response of cultured mitochondria to slight variations in the culture media. Another feature of their behavior is represented by the drawings illustrating conditions observed April 1, 1924. The first vertical column of drawings was made from smears obtained twenty-four hours after subculturing. The second vertical column represents the same subcultures after incubating for twenty days. In many instances, it is evident that the organisms underwent a second pleomorphism during the ageing of the medium. In another set of experiments, the hydrogen-ion concentration of the medium was determined after the organisms had shown a similar second pleomorphism, and it was found that it had changed from an original pH 7.5 to pH 8.8.

The subcultures illustrated at the bottom of the plate under date of March 13, 1924, also show an interesting reaction. The subcultured organisms of 0^{21}–5, which were almost wholly diplococci, were again transplanted after two days into the original kind of medium—nutrient agar. The

organisms that developed in the second subcultures (0^{21}–23—0^{21}–25) were single cocci, more or less similar to the original organism. Smears of these growths eighteen days after transplanting also showed secondary pleomorphism as a result of the ageing of the medium. In this instance, the organisms decreased considerably in size. So also when the organisms of 0^{21}–19 were subcultured into the original kind of medium (nutrient agar) the growth consisted of single cocci. A large number of subculture experiments of this type were made with similar results. It does not appear that this change in morphology was related to the nutritive properties of the substratum because the gross indications of multiplication were identical in all cases.

The subculture experiments demonstrate the extremely sensitive and pleomorphic character of mitochondria. The responses were not only obtained in media of various hydrogen-ion concentrations, but in media with different composition. These results introduce a new factor in the interpretation of the results obtained in the experiments of Mayer, Rathery and Schaeffer, Scott, Nicholson and others. This does not invalidate the interpretations that have been made, namely, that mitochondria are perhaps the first structure in the cell to show a response to abnormal conditions, but the fact that mitochondria are *living organisms* gives to these findings a new significance.

The reality of the bacterial nature of mitochondria introduces a new factor in cytological inquiry which gives promise of rich rewards. The possible relationship of mitochondria to cell functioning has been indicated in numerous researches. These indicated activities have been questioned on the basis of an incompatibility with an *assumed* "passive" nature of mitochondria. The living, bacterial nature of mitochondria introduces a new and firm basis for, at least, a provisional acceptance of the various activities that have been attributed to mitochondria. Obviously, further in-

vestigations in this field, and from the newer point of view, are necessary to establish the full significance of mitochondria in cell activity.

The possible relation of mitochondria to the etiology of disease is immediately indicated by their nature. The researches that have been made in pathology in this connection certainly give promise of ultimate fundamental discoveries. The earlier researches on mitochondria in this field were abandoned when Altmann's "bioblasts" were rejected. The reëstablishment of the fundamental part of Altmann's conception—namely, the bacterial nature of mitochondria, should again serve to stimulate investigations on the relationship of these ever-present microorganisms to disease.

Another feature of the possible behavior of mitochondria in their relationship to the cell may be discussed in a hypothetical way. It appears obvious that there must be a mechanism in the cell that controls mitochondrial growth. Mitochondria, being organisms, would have a tendency to grow indefinitely in a cytoplasmic medium, which is being constantly supplied with proper nutriment. When pathogenic microörganisms invade the cell, various types of responses may be observed. In some cases, the pathogenic organism is of such a nature that it rapidly destroys the invaded cell. In some instances, however, the invading organism is not especially toxic in its reactions, but grows rapidly resulting in the mechanical rupture of the cell. The organism of Rocky Mountain spotted fever produces such a result in the endothelium and muscle cells of blood vessels. The resulting haemorrhagic conditions are responsible for the "spotted" feature of the disease.

The bacteria (*Bacillus radicicola*) in the root nodules of legumes, on the other hand, have the same type of relationship to the nodule cells that mitochondria have to cells in general. When the *Bacillus radicicola* has entered the

root hairs of the legume, certain cells are stimulated to growth and modification into nodule cells. Growth of these cells continues up to a certain point, when not only the cell ceases to grow, but bacterial growth also ceases. When the cells have reached this stage, they are no longer capable of resisting other microörganisms. When one examines an old nodule, microscopically, one usually finds the cells loaded with a great variety of microörganisms, and very few if any *Bacillus radicicola*. It appears evident that the root nodule cells control the growth of the contained bacteria. It further appears probable that the microörganisms in turn exercise an influence upon cell reproduction or division. Eventually, in the growth of the tissue, a state of equilibrium is attained in which the cell and its symbiotic organisms come to a state of rest. Obviously, all living tissues may not develop the same kind of growth mechanism. Many cells in the body stop growing at a certain period in adult life, others, the epithelial cells of the skin, for example, continue to reproduce so long as life is maintained.

The complex changes that chromatin material undergoes in mitosis or cell division, does not appear, on first analysis, to be dependent on influences emanating from the cytoplasm. Obviously, this remarkable series of progressive changes in the chromatin must be dependent upon some stimulus, extrinsic to its own substance. It is conceivable that mitochondria produce chemical substances, or perhaps, physical influences which ultimately become sufficiently strong to inaugurate mitosis. The observations that mitochondria are more numerous in young, growing cells than in old or senile cells, appear to support the hypothesis that mitochondria may be the controlling influence in cell division. However, it would be difficult at present to attempt to definitely correlate the number of mitochondria with cell division. While many observations have been made in which mitochondria appear to be more numerous in young,

growing cells than in mature or senile cells, other observations have been made in which there appears to be no such relationship. The observations of Sokoloff ('25) on neoplastic tissue, also, appear to indicate that the number of mitochondria is unrelated to cell division.

Under abnormal conditions, it is also conceivable that chemical or physical influences may modify the cell in such a manner that the equilibrium is upset, or in other words, the mechanism controlling mitochondrial growth is destroyed. In such an event, the mitochondria would grow and increase so long as food materials are present. Their influence on cell division would, perhaps, be retained, resulting in a continuous growth of cells.

There is a certain amount of evidence that points directly to the probability of the presence of such a growth mechanism in the cell. Long ago, Engler ('82), Winkler ('99), Ernst ('02), Wigand ('84), and others, observed an upgrowth of "bacteria" in intact, but dying algal cells. Some of these observations were made before mitochondria had been discovered. It is quite possible that these "bacteria" were the mitochondria of the normal algal cell which began to grow when vitality ceased in the host cell. The author has obtained similar results in the mitochondrial culture experiments represented in plate II (p. 38). The tissue represented in figure 14 is a small bit of liver tissue that had been incubated only two hours. A comparison of this figure with figure 13, which is the normal unincubated tissue from the same animal, reveals at once a decided increase in the number of mitochondria during two hours incubation. It is hardly probable that this increase in mitochondria was dependent upon the food materials present in the medium, but rather that the growth resulted from the food present in the cell which became available as the cell died. While the piece of liver tissue that was incubated was not more than 6 to 10 mm. in diameter, it does not appear probable

that the food materials of the medium penetrated to the interior of cells in the deeper part of the block in two hours time in sufficient quantity to explain the degree of mitochondrial growth that took place. It should be mentioned that the medium had the consistency of a rather firm jelly.

Another property of mitochondria has been revealed by the culture experiments. This concerns the utilization of urea by the mitochondria of the fetal rabbit liver. The fact that mitochondria may utilize urea suggests a possible mechanism for destruction of urea in the fetus. J. Whitridge Williams ('17), investigated urea elimination in pregnancy, and found that it did not deviate appreciably from the normal. E. C. P. Williams ('23), Hellmuth ('23), and others, have investigated the blood urea during pregnancy, and have found a reduction below the normal. This would appear to indicate, in conjunction with the urea culture experiments, that the mitochondria of the fetus not only utilize the urea produced in the fetal metabolism, but may even extract it from the mother's blood. If it should be found that mitochondria are the stimulating influence in cell division, as it appears possible, then we, apparently, have a tentative explanation for the rapid growth of the fetus. The mitochondria have a special predelection for urea and grow rapidly in its presence, the cells in which they occur undergo frequent divisions with a consequent rapid enlargement of the fetus as a whole. If this property of mitochondria proves to be true in the fetus, then they must change in regard to the character of the food utilized in the adult. That such a change of diet actually occurs has been indicated by the culture experiments.

Dr. K. S. Chouke, working in this laboratory, is investigating the development of the capacity for urea elimination from the kidneys in rabbits. His experiments to date appear to indicate that urea elimination first begins at birth and is gradually increased after birth.

The interest and significance attached to this hypothetical mechanism of cell division becomes more apparent when we realize the bearing it has on the analysis of such uncontrolled growth as we find in neoplastic diseases. Obviously, it will require experimentation and more extensive analysis to determine the reality and nature of this probable growth mechanism. While this hypothesis of the mechanism of cell division may appear to be highly speculative, the variation in mitochondrial content of young and senile cells, together with the above mentioned responses of mitochondria in dying cells, gives reasonable grounds for advancing the hypothesis.

CHAPTER V

Symbionticism

When a biological phenomenon has been observed and the universality of its occurrence has been established, it is within the province of science to correlate it with other biological processes. In attempting to analyze natural processes and principles, it becomes apparent that our knowledge has not advanced sufficiently so that it is possible to make final analyses of natural phenomena, nor ascribe absolute causes to biological activities; nevertheless, our intellectual horizon is broadened if we can reduce a phenomenon to the category of a principle.

The universal presence of microörganisms within the cells of all plants and animals, obviously, points to the consummation of a biologic process or principle as fundamental in significance as that of reproduction. This principle has been named "Symbionticism" by the author (Wallin, '23). Just as sexual reproduction is dependent upon other biological principles or factors (fertilization, cell division), so, also, Symbionticism is the end result of a more primitive factor or principle. This more primitive principle has been named "prototaxis" by the author and has been defined as "the innate tendency of one organism or cell to react in a definite manner to another organism or cell." Prototaxis may be positive or negative. Negative prototaxis is the repulsion of one organism or cell by another. Negative prototaxis can lead to no permanent manifestation or alteration in an organism and consequently it may not be readily detected. Positive prototaxis is the affinity of one organism or cell for another organism or

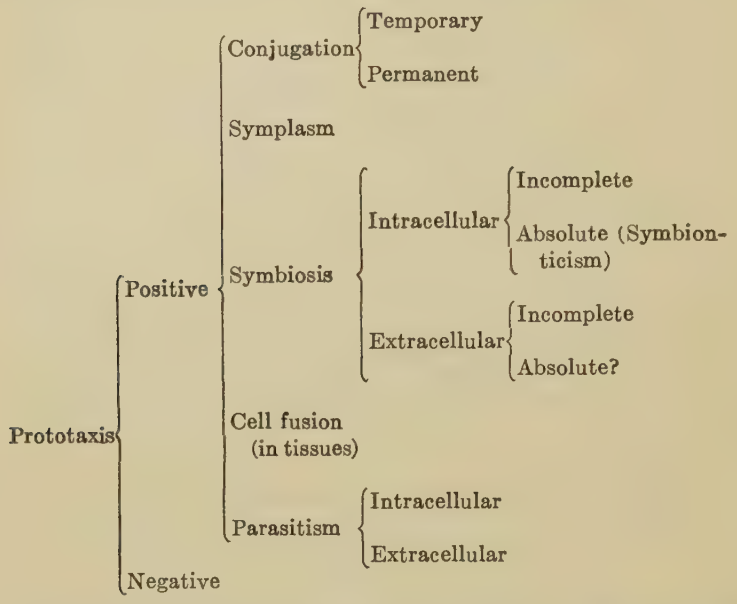
cell. This may result in various kinds of vital relationships. These relationships have long been known and recognized by the terms: conjugation, symplasm, cell fusion, parasitism, and symbiosis.

Prototaxis, on first analysis, may appear to be identical and synonymous with "chemotaxis." It is possible that chemotaxis is the essential force in prototaxis, but doubtless other forces play a rôle in the operation of the principle. It would be quite impossible to analyze and enumerate the factors that enter into prototaxis. It appears probable, however, that such forces as surface tension, light, temperature, moisture, and probably electrical potential are involved in the process. Still other modifying factors may be concerned in the operation of the principle. Our present purpose, however, is not an analysis of the factors involved in prototaxis, but rather a record of its occurrence.

The more familiar expressions of positive prototaxis are to be found in those life relationships that are known as parasitism and symbiosis. It is evident that these two vital relationships are closely akin. The conceptions that have developed in connection with these associations have been based more or less upon the injurious or beneficial results derived therefrom. These conceptions have also involved the idea of purpose. The term "parasitism" implies some benefit to the parasite with either a harmful or indifferent response on the part of the host. Symbiosis is applied to those cases where mutual benefit is derived. It is evident that there is no sharp distinction between parasitism and symbiosis. Considered from the point of view of prototaxis, parasitism and symbiosis are merely different end responses in the expression of one and the same biological principle.

The following diagram may serve to clarify the relation-

ships of the various end results in the expression of the principle of prototaxis:



Conjugation has been observed, chiefly, in the protozoa or one-celled animals. It consists of a temporary, in some cases permanent, union of two individuals of a species. In the paramecium, for example, two individuals come together and remain attached for a short period of time. During this attachment certain internal changes occur, and an interchange of some materials of the two conjugating individuals takes place. Conjugation in the bacteria has been described by Löhnis ('21) and others. On account of the small size and the mode of life of the bacteria, conjugation in these forms is not so readily detected as in the larger protozoa. Löhnis has submitted a number of photographic illustrations of bacteria in the state of conjugation to support his claim that bacterial conjugation does occur.

"Symplasm" is a term proposed by Löhnis and Smith for another phase of bacterial behavior. It represents a stage in the various life-cycles of bacteria. Briefly, it may be described as a clumping together of a number of bacteria of a single strain in which the boundaries of the individual organisms are lost. The bacteria coalesce and resolve into an amorphous mass; this constitutes the symplasm. Later, in the completion of the life cycle, individual bacteria are reformed by a dissolution of the symplasm. Löhnis has collected a considerable amount of evidence from the literature to support his own researches on this subject. Almquist ('22), and others, also, have observed the symplasm stage in the life cycles of certain bacteria.

Cell fusion represents another type of expression of prototaxis. The end result in this type of activity may be represented by two kinds of cell relationships. In one case, cells appear to "flow together" and produce a true syncytium. The author observed a type of fusion in the branchial epithelium of the lamprey larva in which the cell boundaries completely disappeared (Wallin, '18). The other type of cell relationship is found in certain tissues in which the cells have protoplasmic bridges extending from one to another.

The interesting experiments of Galtsoff ('25) on "Regeneration after dissociation" of the cells of the sponge, in which "the regeneration is primarily the process of coming together of cells, accompanied by their further differentiation," demonstrates another phase of the same principle.

In symbiosis two dissimilar organisms become intimately associated. This type of vital relationship was, apparently, first observed by Reinke in 1872, who named the phenomenon "consortism." In 1879, De Bary observed and recorded the same phenomenon, and introduced the term "symbiosis," which has since been adopted into common usage.

Since the observations of Reinke and De Bary, the number of recorded cases of symbiosis has steadily grown. In most cases of symbiosis early described the two symbionts were multicellular plants or animals. It is perhaps because of this circumstance that symbiosis has been looked upon more or less as a digression in plant and animal behavior. That symbiosis may represent the end result of a biological principle appears to have escaped detection. The kind of symbiosis in which one symbiont is a microorganism is not so well known. It is this type of symbiosis, which we designate "microsymbiosis," that is of particular significance in the theory of Symbionticism.

Various classifications of symbiosis have been devised by different authors. These have been based for the most part upon the physiological responses of multi-cellular symbionts. While such classifications may be valuable from one point of view, they are of little significance in connection with prototaxis. Based upon the morpholgic relationship of the symbionts, symbiosis may be divided into intracellular and extracellular types. Symbiosis may be incomplete or absolute (facultative or obligate) depending upon the degree of physiological dependence of the two symbionts. In incomplete symbiosis, the two symbionts are capable of more or less independent existence and reproduction. In absolute symbiosis, they are incapable of independent existence and reproduction. In this grouping the author has particular reference to microsymbiosis. It might be well to use the term "macrosymbiosis" to designate that type of association in which the two symbionts are multicellular organisms. In macrosymbiosis, the two symbionts are not so intimately associated, and appear to have little or no significance in the modification of species.

The term "symbiosis" is used in bacteriology in a different sense than in general biology. Certain bacteria, ap-

parently, grow only in the presence of certain other bacteria. One organism produces extrinsic conditions that are essential for the life of another. Their relationship is more in the nature of an *indispensable association* and has nothing to do with prototaxis. It is possible that bacteria may also play the rôle of relative or host symbiont. The D'Herelle phenomenon would allow of such a possibility.

Mitochondria represent one of the symbionts in the expression of a positive prototaxis resulting in an absolute symbiosis. The universal presence of mitochondria in the cells of all plants and animals constitutes evidence that the development of such relationships is not a digression from normal biological behavior, but rather the result of a fundamental principle. The universality of positive prototaxis resulting in absolute symbiosis, or Symbionticism, forces us to recognize this as of fundamental importance. Just as reproduction insures the *perpetuation of existing species*, the author believes that *Symbionticism insures the origin of new species.*

In absolute symbiosis, the adjustment responses of the two symbionts have been completed, making it difficult to recognize their exact nature. On the part of the bacterial symbiont, it appears, however, that one response has been the development of fragility. While it is quite impossible in most cases to recognize the complete modifications that have occurred in the host cell in absolute symbiosis, modifications are specifically indicated in many of those that are as yet incomplete.

It is recognized that the ideas on symbiosis embodied in this book are at variance with those usually conveyed by this term. It is perhaps unfortunate to utilize an established terminology with a new meaning as it may lead to confusion. Nevertheless, the author believes that in this

instance it lends itself to greater ultimate clarity to enlarge and re-define the significance of a term, rather than introduce an entirely new word to replace the old.

A number of authors (Buchner, Nuttall, Cleveland, Meyer and others) have recently discussed the propriety of the term "symbiosis" in connection with particular life relationships. These discussions have been based upon the original definition of symbiosis as given by De Bary, in which the idea of *mutual* advantage is implied. Other writers (Geddes, Thomson, Farmer, Wallin, Castellani and others) appear to have sensed an underlying principle in the various types of life relationships, and do not draw a sharp distinction between symbiosis and parasitism.

If we should retain the narrower conception of "mutual advantage" in symbiosis, then it follows that we must limit the term to those *chance* relationships in which "benefit" happens to be an accompaniment to both symbionts. It is often most difficult to determine the nature of the advantage gained by a symbiont. From the recent discussions on symbiosis one gathers the impression that there is an *element of choice* exercised by the symbionts. Thus to quote Meyer ('25),

> From the experiments and the findings reported, it is easy to assume that the mollusks derive some benefit from the intracellular bacteria as anabolists or catabolists of metabolic waste products, but, what possible benefit can the microörganism derive from the association? . . . . The recent literature on "symbiosis" has been carefully searched for other examples, but only theories have been found which are not established on any scientific basis. The function of the microscopic "symbiotes" and their benefit to the host are explained, but little or nothing is said regarding the possible advantages to the microörganisms. . . . . Buchner's suggestion that the intracellular organisms are benefited by being protected within the host from the drastic atmospheric influences of heat, cold, desiccation, etc., is a trifle unreasonable.

It appears obvious that the end responses in life relationships are not the *fundamental cause* for these associations, but rather the chance outcome which is dependent upon the physico-chemical properties and reactions of the symbionts concerned. Certainly, one can not recognize a display of *choice* in microörganisms which would lead them to seek out certain hosts on account of advantageous qualities; nor can we recognize such properties in the host. The cause for the development of a life relationship is unrelated to the responses accompanying the association. It is these circumstances that led the author to introduce the term "prototaxis" to explain the "cause" for the development of life relationships, and the term "Symbionticism" to designate the *fundamental tendency and object* of prototaxis.

In attempting to explain the nature of the development of symbiotic complexes, Meyer states:

> During an early period, the bacteria, fungi, etc., now found as harmless entities within the insects, are probably parasites producing pathologic conditions and disease. Acquired immunity later becomes inherited, and the microörganisms are gradually gotten under control. The insects do not rid themselves of the invaders on account of the fact that transmission from generation to generation is established with remarkable precision. Still later, the invaders lost all of their harmful effects, and since they secrete enzymes that prove serviceable to the hosts, the conflict ends in mutual adaptation; just as in plants (orchids and their mycorhizas) the symbiosis between the tissues of the host and the microörganisms is a phenomenon of parasitism, infection or disease, which has finally become essential to the existence of the animal.

In a similar manner Cleveland ('26) suggests that "Such structures as mycetocytes, bacteriocytes and mycetomes of insects may be the survival of profound pathological changes."

While it might be true that some symbiotic complexes may have arisen in this manner, there is no evidence at hand to indicate that this is the usual manner of development of symbiotic complexes. On the basis of our knowledge of microbial behavior, we must admit that there exists a wide range of reactions of living organisms to each other. We are justified in concluding that in many instances the physico-chemical properties of the symbionts entering into a relationship are of such a nature that the terms "parasitism," "infection" and "disease," as they are commonly understood, could not be applied to the relationship.

The evidence for and the nature of the development of symbiotic complexes are discussed at more length in the chapters following.

CHAPTER VI

Microsymbiosis

Long before it was discovered that bacteria enter into symbiotic relations with plants and animals, it was known that single-celled plants, particularly the algae, may be symbiotic guests in the tissues of certain animals. Due to their larger size and more distinctive characteristics, the algae are more readily detected in symbiosis than bacteria, and consequently they are better known in microsymbiosis. The morphologic and physiologic responses that are indicated in these symbioses vary within wide limits. The "degree" of symbiosis also varies. This variation extends all the way from an occasional presence of the symbiont to absolute symbioses. The study of microsymbiosis has been pursued by a number of investigators during the past twenty-five years. More recently Paul Buchner (1921) has rendered an invaluable service to investigators by his comprehensive and analytical review of the literature. A considerable portion of the data which will be catalogued in this chapter has been taken from Buchner's treatise. The reader who desires more detailed descriptions than will be found here is referred to this comprehensive monograph (in German).

The study of microsymbiosis, perhaps, had its beginning in the interest aroused in the nature of the chlorophyl that occurs in a number of lowly animals. Quite a few of these animals are familiar to the general biologist on account of their green coloration (*amoeba viridis*, fresh water sponges, *hydra viridis*, and other coelenterates and marine worms). For a long time the green color was not associated with chlorophyl. Later, discussion arose as to its nature and

origin. Siebold in 1849 declared that if the green material of animal cells is not identical with chlorophyl, it is, at least, very closely related to it. Max Schultze, a little later, by means of chemical tests, definitely established the similarity of plant and animal chlorophyl. The question then turned to the origin of chlorophyl in the animal cell. A number of investigators (Geddes, Lankester, Kleinenberg, McMunn, and others), declared for an animal origin of the chlorophyl in the cells of animals. The bearing that this interpretation had upon the much discussed "differences" between plants and animals, stimulated a large number of biologists to investigate the problem. Cienkowsky, in 1871, was the first investigator to present evidence of the "parasitic" nature of the chlorophyl bodies. He described the cellular nature of these bodies with their nuclei, starch granules, and cell membrane. He also observed that the chlorophyl bodies continued to live after the death of the animal host. R. Hertwig, Brandt and later Geddes, brought forth evidence in favor of the symbiotic nature of the chlorophyl bodies. They were shown to be symbiotic algae. Brandt named the green forms "Zoochlorellae" and the yellow-brown bodies, "Zooxanthellae."

Microsymbiosis in which single-celled plants are the definitive symbionts, apparently, has not persisted in the higher groups of animals. It would appear that nature stopped experimenting in this direction long before the higher mammalia came into existence. Still it appears probable that the fruits of these "experiments" have not been entirely lost to the higher animals. The similarity of chlorophyl and hemoglobin is suggestive of a possible origin of hemoglobin from chlorophyl or a closely related substance. This possibility is particularly enhanced when it is viewed in the light of Symbionticism. However, on account of their greater familiarity and the known physio-

logical responses accompanying their invasions into animal cells, algal symbioses furnish important evidence of the proposed mechanism by which Symbionticism operates in the origin of new species. Furthermore, that type of symbiosis in which algae are the definitive symbionts lends itself to experimental analysis more readily than most cases of bacterial microsymbiosis. So far as Symbionticism is concerned, there is no fundamental difference in bacterial and algal symbiosis. Both types of symbionts, as well as symbiosis in which other types of organisms are definitive symbionts, will be considered in the following review.

The cases of symbiosis in which bacteria are the definitive symbionts, and which will be discussed in this chapter, obviously, represent only those instances in which the bacterial nature of the symbionts have been readily recognized. It has been suggested in a few instances, that the bodies in question are mitochondria. Such confusion is rather to be expected when we realize that mitochondria and bacteria in general are one and the same thing, but showing wide variations in morphology, physiologic response, staining reactions, and responses to physical and chemical agents.

Symbiosis in the protozoa, or one-celled animals, has been studied mainly in the fresh-water forms, although a few marine species are known to contain symbionts. The majority of described cases of symbiosis in this phylum deal with the more readily detectable algal symbionts, of which there are numerous examples. Buchner divides the protozoa into three groups from the standpoint of algal symbiosis. The first group contains algal symbionts in whatever locality they may be found. The second group he calls "facultative algae breeders." This group is made up of those species which contain algal symbionts in certain localities, or under certain conditions. The third group is made up of all remaining protozoa, and is characterized as "algal enemies;" they never harbor algal symbionts.

The "facultative algae breeders" are interesting and significant in our study of microsymbiosis, in-as-much-as they, apparently, furnished evidence of the influence of extrinsic factors in the development of symbiosis. It is possible that the particular species that fall in this group are represented by *varieties* in different localities, and that they do not show any distinctive morphological features that differentiate them from the type species. Such "varieties" may have chemical and physiological variations which could only be detected by experimental methods. It is also possible that the microsymbiont may have different properties in different localities. It appears more probable, however, that the absence of the algal symbionts in certain localities is due to extrinsic factors, such as temperature, oxygen, light, pressure, etc. Certain cases of symbiosis, which will be mentioned later, furnish evidence of the influence of extrinsic factors.

The hereditary transmission of the algal symbionts is a simple process in the protozoa, where reproduction consists of simple fission of the parent organism. When the cytoplasm of the protozoan host divides, the algal symbionts are distributed more or less equally between each of the daughter animals. In the encystment stage of the protozoan host, it has been observed by Penard ('90) and Leidy ('79) that in a number of the *Foraminifera* the algae are retained within the body of the encysted animal. Gruber ('04) also found algae within the cysts of *Amoeba viridis* that had been sent from America to Germany. In some protozoa, however, the algal symbionts are expelled from the body of the host before or during encystment (*Acanthocystis aculeata*), but the symbionts are retained in the wall of the cyst.

A number of investigators have observed that when the host is in a state of starvation, the algal symbionts are digested by the host animal. Greef ('75) observed a spon-

taneous expulsion of the symbionts in *Acanthocystis*, in the vegetative stage and a subsequent re-entrance of the symbionts into the animal body.

Seasonal variations in the occurrence of algae in a host have been described by Balbiani, Roux, and Wesenberg-Lund. Roux observed that *Stentor polymorphous* harbored algae in the spring of the year, while in August, September and October, the animals did not contain any algae. The experiments of LeDantec ('92) are significant in connection with a study of the physiological responses under the influence of extrinsic factors. He placed *Paramecium bursaria* in the dark, andfound that after twenty-four hours the algae had become brown in color. When kept there for a longer period, the algae were found to be in a state of digestion. When the animal is later returned to the light and kept in sterile water, the algae do not reappear; it is necessary for a reinfection to take place.

An interesting example of the apparent influence of extrinsic factors on symbiosis is to be found in the well known luminiferous *Noctiluca milearis*. This animal is quite widely distributed, and usually does not contain any algal symbionts. M. and A. Weber ('90–'91) found this animal in the Bay of Bima on the Island of Sumbawa (Indian Ocean) so numerous and so filled with green algae that the surface of the water appeared green. They could find no evidence of digestion of the symbionts.

Zooxanthellae, or "yellow algae," have been described in a large number of protozoa. These forms have been observed to produce "spores" while in the symbiotic state. These spores are apparently retained in the cytoplasm of the host as small yellow or brown granules. Zooxanthellae in the free-living state are often provided with two flagella at one end of the body. These flagella are left behind when the yellow algae enter the body of a host.

Bacterial symbionts in the protozoa have been described

only in one genus, *Pelomyxa*. The bacteria are rod-shaped and vary in size in the different species. In *Pelomyxa vivipara* they are grouped close to the nucleus, reminding one of the oft-observed arrangement of mitochondria. In other species they are scattered throughout the cytoplasm just as mitochondria often are. These symbiotic bacteria measure 10–15 micra in length in some species, while in other species they may be from 40–50 micra long. The physiologic significance of these symbionts has not been determined.

In the Sponges, algal symbiosis is fairly common, particularly in the fresh-water species. More than one species of algae may be present as symbionts. Some sponges contain only single-celled algae within their cells; others harbor the more complex multicellular forms. In some species of sponges, the symbionts are represented by a varying algal flora. The physiologic and morphologic responses in these symbiotic relationships do not appear to have been investigated. No reference to bacterial symbionts in the sponges has been found.

Symbiosis in the Coelenterates has been widely known and studied through familiarity with the fresh water form *Hydra viridis*. Buchner gives a table of the species in which symbiosis has been recognized. The list includes a large number of species of *Hydrozoa*, *Scyphozoa*, *Anthozoa* and *Ctenophora*. The definitive symbionts in all these symbiotic relationships are zoochlorellae and zooxanthellae (green and yellow algae). In the more simple *Hydrozoa* the symbiotic algae are located chiefly in the entoderm. There is a variation, however, in their distribution within this layer. The symbionts are usually absent in the lower part of the entodermal cavity where the cells are vacuolated. They are present, in most cases, in the entoderm of the tentacles. In the higher coelenterates the symbionts are more widely distributed. They are also

present in the ectoderm in some species. In some cases there appears to be a definite localization of the symbionts in a particular part of a tissue. Thus, in the canals of the so-called liver of the Portuguese man-of-war the algal symbionts are only present in the upper cells of the canals. These cells do not have the typical entoderm-cell appearance, while those in the lower half of the canals suggest secretory and absorptive functions. The symbionts are also found in other parts of this animal. When the animal reproduces by budding, the daughter forms receive the algal symbionts in a passive manner. The number of symbionts present in a cell varies considerably in different species. The usual number is four to eight, but in some cases it runs up into the hundreds.

An idea of the intimacy of the relation between the algae and the host in some instances is suggested by the ease with which the former can be eliminated when the host is kept for a time in a dilute solution of glycerine. Glycerine is known to be highly toxic to many bacteria and other low forms of life with a notable exception of certain filterable viruses whereas it has little or no effect upon higher forms. It is supposed that this effect is produced largely through its influence upon osmotic pressure within the cell.

The distribution of the symbionts in a hexacoral (*Adamsia diaphana*) described by K. C. Schneider ('02) is also interesting and indicative of the specificity of certain cells to the symbiont. The symbionts are found in special cells in the septa which produce a bulging at that point. These special cells have a morphology which differs from that of the other cells of the septum. This appears to be the lowliest animal in which there has been found to be a distinct morphologic response in the host to the presence of the symbiont.

The behavior of the algal symbionts in sexual reproduc-

tion varies in different Coelenterates. In a number of species, the symbionts do not enter the egg. In such cases, the later larval stages become infected from the surrounding water. In the hydroids, it has been observed by a number of investigators that the algal symbionts are carried from one generation to another within the ovum. Hamann ('82) studied egg formation in *Hydra viridis* and observed the algal symbionts transferred to the egg. He believed that there was no active movement of the algae into the eggs, but that they were carried passively in the "food stream" from the entoderm to the egg in the formation of the yolk. Such an interpretation, it appears to me, is a rather superficial one. The experiments of Hadzi ('06) on the influence of light on egg infection are interesting and significant. *Hydra viridis* was kept in the dark during ova formation. The ova developed under these conditions, but more slowly and they were colorless. Out of twenty eggs produced in this manner, only one developed into a polyp. This polyp was also colorless, and lived but a short time. He also tested the influence of colored light on egg development. In red light the eggs developed into normal polyps, and contained the algal symbiont. In blue and violet light a few individuals developed into polyps, while in weak green light no development was observed.

Beijerinck ('04) discovered a free living alga that was strikingly similar to Brandt's zoochlorellae (symbionts). He inoculated some of these algae into a colorless *Stentor polymorphus*. Vacuoles formed around the algae, and in a short time they disappeared apparently as a result of digestion. Experiments of a similar nature have been made by various investigators with identical results. These experiments indicate that there is a distinct "specificity" of the organisms concerned in symbiosis. They also indicate the presence of chemical or unseen differences in organisms with similar morphology.

Symbioses in the turbellarian worms have been described in the following groups: *Acoela*, *Rhaddocoela* and *Alloeocoela*. The definitive symbionts in all cases are green and brown algae. The thorough and extensive researches of Keeble ('10) on the two marine species, *Convoluta roscoffensis* and *C. paradoxa*, represent one of the more extensive and complete analysis of microsymbiosis that has come to my notice. The observations and experiments of Keeble were conducted over a period of ten years. His results have been confirmed by other investigators. In his book "Plant-Animals, a Study in Symbiosis" he describes in detail the habitat and habits of these two species. He also describes a number of experiments determining the reactions of the worms to various extrinsic influences. These are valuable studies in animal behavior, but since they have no direct bearing on our problem they will not be further considered.

Convoluta roscoffensis contains green algae and *C. paradoxa* harbors yellow-brown algae. In the adult condition *C. roscoffensis* ingests no solid food. Kept under abnormal conditions, Keeble found that the animal may become cannibalistic. *C. paradoxa*, on the other hand, is a voracious feeder throughout life. The adult *C. roscoffensis* depends upon the carbohydrate food supplied by the symbiotic algae within its tissues. Obviously, the time must arrive when there is nitrogen deficiency, and instead of obtaining this from the outside, it digests the algal symbionts that had previously nourished it. Soon after the destruction of the symbionts, the animal dies. In the early stages of development, *C. roscoffensis* contains no algae, but feeds like most animals upon other animals and plants. Then for a time it receives food from two sources—from ingested plants and animals and from its own green cells. Ultimately, it depends upon the algae alone, and thereby brings about its own destruction.

The two worms are remarkable among the turbellarians in possessing no excretory system. The nitrogenous waste products are stored in the tissues of the animal. In the cells of young worms which do not yet possess any algal symbionts, crystalline bodies can be seen. These cells remind one of the "urate cells" of insects. After the algae have invaded the tissues of the worms, these crystals disappear, and in adult life they are absent. "Comparative cultures of the free stage of the infecting organism have demonstrated that the algae flourish better when supplied with nitrogen in the form of uric acid than when it is supplied with a nitrate (potassium nitrate)."

The transmission of the symbionts in the reproduction of the host does not follow in the manner that one might expect in such cases of obligate symbiosis. The egg cell itself does not contain any symbionts, but the egg capsule was found to harbor the algae. It is only in a later developmental stage that the offspring become infected.

The host in one of these symbiotic associations (*C. roscoffensis*) exhibits definite physiological and morphological responses to the invasion of the symbiont. The cessation of food ingestion from the outside apparently develops into a fixed physiological habit. The failure in the development of an excretory system appears to be an evident morphologic variation associated with the anticipated presence of uric acid-consuming symbionts. Keeble says "the conclusion forces itself upon us that the green and yellow-brown cells in the bodies of their respective hosts obtain access to and utilize the stores of waste nitrogen compounds accumulated therein. Or, to put the same idea in another way, green cells and yellow-brown cells constitute the excretory organs of *C. roscoffensis* and of *C. paradoxa* respectively." Keeble suggests the possibility that the ultimate death of the animal may not be due to nitrogen starvation, but to "an aggravated attack of 'uric acid trouble.' "

The responses shown by the algal symbionts are equally interesting and significant. The free-living algae possess a nucleus, besides pyrenoid and chloroplast. After the algae have become symbionts they gradually lose their nuclei. "A parallel suggests itself between the green cells of *C. roscoffensis* and the red blood corpuscles of the higher vertebrates. As the red discs are enucleate, partial cells budded off from the nucleate red cells, so may the green cells be regarded as enucleate, partial cells budded off from the outermost, nucleated green cells; and as the red blood corpuscles are of limited life and specialized (respiratory) function, so are the green cells of *C. roscoffensis* of limited life and specialized photosynthetic function." This statement, perhaps, does not convey the full significance of the status of the algal symbionts. The morphological and physiological responses indicated in algal symbionts will be discussed in more detail in another chapter.

Algal symbionts in the annelid worms, apparently, have never been observed. Bacterial symbionts, however, have been described by a number of authors. These symbionts have been called "bacteroids," and they have been credited with connective tissue fibrillae formation. Cerfontaine ('90) discovered these bacteria in the coelom of the dew worm, and believed that they are the cause of the rapid putrefaction that takes place in these animals after death. Cuenot ('92) and K. C. Schneider ('02) investigated these organisms and confirmed Cerfontaine's diagnosis of their bacterial nature. Trojan ('19) investigated the bacteria and observed their relationship to fibrillae formation, but believed that they were "chromidia" derived from the nucleus. Schneider, also, observed these bacteria in *Nereis, Sigalion* and in marine polychaetes. Buchner emphasizes the difficulty of distinguishing between bacteria, mitochondria and mitochondria-like cell-inclusions.

In the Bryozoa, yellow algae have been described in

Zoobothrium pellucidum by Brandt ('83). Brandt, also, described yellow-brown amoeboid algae in the echinoderms (*Echinocardium*). Geddes ('79) described brown amoeboid bodies in various echinoderms. Holothurian larvae also are supposed to contain yellow algae, present as clumps. Brandt observed that when the larva dies the color disappears at once. The bodies can be demonstrated after death however by means of iodine. Semon raised the question whether they are algae or pigment cells. Vogt and Jung ('88–'89) have described algae in the crinoids. De Negri ('76) claimed that *Elysia viridis*, a mollusc, contains real chlorophyl. Brandt investigated this form, and found symbiotic algae present in the contractile canal system.

In the land snail, *Cyclostoma elegans*, there is a peculiar group of cells located between the kidney and the stomach that supposedly functions as a "storage kidney." Mercier ('13) investigated this structure, and demonstrated the presence of bacteria in the cells. In the early stages of development the cells contain no bacteria. A peculiar type of inclusion (like a cyst) develops in the cells, and at the same time bacteria appear in the cytoplasm. The "cyst" contains uric acid and xanthin. It enlarges until there is only a thin layer of cytoplasm around it. The bacteria also increase in number. They vary in morphology; some are straight rods; others slightly S-shaped, and the remainder are filamentous. Presently, phagocytes wander in amongst the cells and later penetrate them. Vacuoles then develop in the phagocytes and the bacteria can now be seen clumped in the vacuoles, apparently, in a state of digestion. The uric acid cyst dissolves and is absorbed. Mercier found this structure in animals obtained from various localities. He was not able to find a mature female with eggs, and consequently was unable to study the manner of transmission of the microsymbionts.

More recently, Meyer ('25) has investigated *Cyclostoma elegans* and a number of related species. He was able to cultivate bacteria from the animals, but was not convinced of their relationship to the bacteria in the "purinocytes."

In the small group of the *Ascidia* (*Molgulidii*) urate-storage kidneys are also found. These storage cells contain a fungus in place of the bacteria, according to Buchner. They produce both mycelia and spores.

Microsymbiosis is very widely distributed in the insects, being found in all the orders of this group. The definitive symbionts are bacteria in a large number of cases. We shall discuss here only a few of the more representative cases, and such relationships as have a more intimate bearing on Symbionticism.

All of the species in the family Blattidii harbor symbiontic bacteria. Blochmann in 1887 was the first to describe bacterial symbionts in these insects. In *Blatta germanica* and *Periplaneta orientalis* he found that the central part of the fat bodies was composed of cells devoid of fat. These cells contain small rod-shaped bodies which were of undoubted bacterial nature. When he failed in his attempt to cultivate them, he concluded that this was evidence of their intimate dependence upon the insect organization. Cuenot ('92), Prenant and Henneguy ('04) investigated these cells in the fat bodies, and believed that the rod-shaped structures represent metabolic products. Mercier ('06) was able to cultivate them outside of the body and definitely established their bacterial nature. Frankel, a pupil of Buchner, investigated a large number of species, and demonstrated the presence of symbiotic bacteria in all. Buchner proposed the name "Bacteriocytes" for all cells containing bacteria. The bacteriocytes vary in shape and arrangement in different species. The bacteria also vary in size in the bacteriocytes of different species. Philiptschenko ('07) studied these insects, and showed that

the bacteriocytes in young animals also contain glycogen. In older animals he found only fat and uric acid concretions.

Mercier investigated the bacteriocytes after starvation, and found morphologic variations in the bacteria, apparently due to inanition. In an animal that was infected with a yeast-like parasite, the bacteriocytes were not present.

Blochmann, Mercier, Buchner, and Frankel have shown that the bacteria are transmitted from one generation to another in the egg cell. Heymons ('95) studied the bacterial symbionts in embryonic development, and demonstrated their presence in all stages.

Symbionts in ants were first observed by Blochmann ('84–'87). In the midgut of *Camponotus ligniperda*, peculiar cells filled with bacteria-like rods are constantly present. So also in *Formica fusca* there are two cell-groups of similar appearance near the ovaries. He also observed the bacteria-like rods in the ova. Strindberg ('13) believed the rod-shaped structures to be mitochondria. Buchner investigated these bodies and concluded that they were fungi. The cells in which they are present he calls "mycetocytes." The development of the ova and the infection of the egg have been clearly described by Buchner. After the ova have entered the oviduct and the nurse cells have come into position, the symbiotic rod-shaped fungi begin to enter the follicle cells. A little later they penetrate the ova as well as the nurse cells. When the ovum has increased to about three times its original size, the follicle cells contain but few symbionts, but the ovum is so packed with them that the cytoplasm of the egg cell is obscured. The host-cell plasm now increases in amount, and at the same time appears to have assumed a control over the growth of the symbionts, for they increase no further. Buchner believes that this is a chemical regulation. A little later the yolk begins to accumulate in the egg cell. During this process

the symbionts form a closely packed group at one pole of the egg. This process reminds one somewhat of the nebenkern formation, as described by Bowen and others. The nucleus of the ovum develops a peculiar activity during the early stages of the symbiont migration into the egg. Parts of the nucleus appear to be thrown off into the cytoplasm so that the ovum appears to become multinucleate. Buchner believes that these bodies originate from the nucleolus.

Mycetocytes are also to be found in the wood-boring insects. Buchner investigated some of these forms, and gives a comprehensive description of their relationships and the mode of transmission of the symbionts. In *Litodrepa panicea* there are peculiar rounded outpocketings of the foregut, which are composed of mycetocytes with a few compressed entodermal cells. These mycetocytes contain symbiotic yeast cells, which are oval in outline and usually contain a large vacuole. Escherich ('00) was able to cultivate them in artificial culture media. The eggs of the host do not contain the yeast symbionts, but Buchner found them clinging to the surface of the egg capsule.

Buchner discusses the physiological significance of the symbiotic yeasts in these animals. A primitive type of gland organ is indicated by the outpocketings of the gut tube. The digesting food mass or chyme lodges in these pockets. He believes that the yeast cells of the mycetocytes produce enzymes which are secreted into the gut lumen, and act as ferments digesting the food for the insect. Such an interpretation, he mentions, is strengthened by a similar utilization of microörganisms in the digestion of cellulose in the herbivora. In these higher animals, the cellulose-digesting bacteria are not intracellular symbionts. They are free in the lumen of the gut tube.

It is important to note that a large number of insect

species, if not all of them, harbor free bacteria in their intestines. It is a difficult matter to determine definitely the physiological significance of bacteria in such small animals. Buchner describes the free bacterial symbionts in one insect, the olive fly, (*Dacus olae*), which deserves consideration. The description of the symbiosis in this species was made by Petri ('09). In the larva of this form which feeds on the olive, there are four large outpocketings in the midgut. These pockets are lined with a flattened epithelium. The lumen of the pockets is completely filled with bacteria. When the larva transforms into the pupa, some radical changes develop in the gut tube. With the cessation of feeding, the bacteria leave the pockets in masses, and pass through the remaining part of the midgut and the hind-gut to the outside. A few bacteria remain in the alimentary canal. They pass forward into the foregut. During the transformations of the pupal stage, an unpaired dorsal, gland-like outpocketing develops in the pharynx. In this structure the bacteria find a haven. They persist in this situation in the imago, or adult. Two strains of bacteria have been recognized in the diverticula of the olive fly. Paoli ('08) discovered two strains of bacteria in the irregularly-arranged tubercules on the branches of the olive tree, which he thought were identical with the symbionts in the olive fly.

There still remains a large number of symbiotic relationships in the insects that have been described in literature. In a few instances the symbionts are associated with distinct organs. For example, in certain lice, a "mycetome," richly supplied with tracheal tubes, is present in the abdomen. Certain insects harbor *distinctive* bacteria (luminous); these cases will be left for later consideration.

In a number of insects and insect-like organisms (ticks and other arachnoids), small bacteria-like bodies are often present. While the nature of these bodies is not definitely

known, it has been demonstrated that they are capable of producing disease in man and some of the higher animals, when introduced through bites or other forms of injury. These organisms are known collectively as "Rickettsia bodies." The Rickettsia bodies of the wood tick (*Dermacentor venustus*) are well known through the researches of Wolbach ('19), and many others. The microörganism appears to be an occasional parasite of the wood tick, since all individuals do not seem to harbor them. In so far as can be learned, they produce no injurious effects in the larva, nymph or adult wood tick. They have been demonstrated in the egg of the host. The particular disease in man associated with the microörganism is known as "Rocky Mountain spotted fever," and is more or less limited to certain sections of Montana. The host, however, is widely distributed over the Rocky Mountain region. Dr. F. E. Becker, working in this laboratory, has been able to demonstrate structures indistinguishable from these Rickettsia bodies in wood ticks that were collected near Boulder, Colorado. He also has examined the tissues of a number of guinea pigs upon which the native wood ticks have been placed. In the tissues of these guinea pigs he was able to demonstrate the Rickettsia-like bodies, but in a different situation from that affected by the Montana strain. The Montana strain, which produces Rocky Mountain spotted fever, localizes in the peripheral blood vessels. The Colorado strain, according to Becker's findings, appears to select the intestinal tract. It appears probable that the Rickettsia bodies are symbiotic microorganisms in the wood tick, that vary in their specific reactions in different localities. These organisms are extremely delicate, and difficult to demonstrate on a microscopical slide, due to their great fragility.

In the examples of microsymbiosis that have been reviewed in the preceding pages, a large number of the defin-

itive symbionts were algae, fungi or yeasts. Recorded instances of bacterial symbiosis have not been so numerous as one might expect. Various reasons may be given for this *apparent* scarity of bacterial symbionts. Unless the bacterial symbiont possesses definite stability, or has distinctive characteristics, it would not be detected readily within the cell. Furthermore, when bacteria are present in an intracellular position, they are usually called "mitochondria" and their bacterial nature has not been generally recognized.

Certain bacteria, however, have physical characteristics as distinctive as have algae or yeast cells which may readily be recognized in microsymbiosis. Among these bacteria are the "phosphorescent" or luminiferous forms which have been observed in symbiotic relationship in a large number of animals. The biological relationships exhibited by these symbionts in respect to their hosts, furnish perhaps one of the most significant chapters in microsymbiosis. On account of their luminiferous properties, these organisms can be "followed and watched," not only in the development of the host, but also in experimental investigations. This can not be done so easily nor with the same degree of accuracy and certainty with microsymbionts that do not have such outstanding characteristics. We are again indebted to Buchner for an extensive review of the literature on "Light Symbiosis," and for original observations in this important branch of microsymbiosis.

The question of the nature of the material that is luminous in plants and animals was formerly the subject of considerable polemic discussion. Certain kinds of decaying woods are known to emit light of a continuous nature. Bacteriologists long ago demonstrated that the emission of light in these cases is associated with bacteria, apparently feeding on the decaying wood. In the common "lightning bug" or "firefly," on the other hand, the light appears

to be intermittent in character. An anatomical investigation of the "lightning bug" reveals a definite body or organ in the abdomen that is associated with the production of light. This organ is supplied with branches from the tracheal respiratory system, as well as with blood vessels. It has been thought that the cells of this organ contain a material of fatty nature, sometimes called "noctilucine" or "luciferin," which when oxidized produces light. The intermittent character of the light was supposed to be produced by contractions and relaxations of the tracheal tubes under the influence of nerves. Dubois ('86, '87) found that the eggs in two familiar families of beetles, while still in the oviduct and before fertilization had taken place, were luminous. He also noticed that after the egg had been laid it retained luminescence a much longer time than did segregated bits of the luminiferous organ. Dubois saw minute granulations in the cytoplasm of the luminiferous organ, as well as in the egg cell. These granulations which he observed increasing by division, he also found to be associated with luminescence. He named the granules "Vacuoloids," and considered them as elementary and highly significant parts of the living substance which might be bearers of a number of other functions.

Regarding the finer structure of the luminferous organs in the beetles, there are two types of cells entering into the formation of the organ, a deeper opaque and a superficial layer. The opaque layer is made up of cells filled with urates, which performs a secondary function in the organ, acting as a condensor and reflector. The superficial layer of cells is transparent and represents a modified portion of the chitin cells. According to M. Schultze ('65), Dubois and others, it is only the superficial cells that contain the luminous substance. Pierantoni, in 1914, demonstrated the presence of two kinds of bacteria in the cells. One variety is rod-shaped and about 10 micra in length. The

other variety is a form of coccus. He was able to cultivate them in an agar-peptone medium in which they were collectively colored light yellow and were opalescent.

The bacteria that developed in Pierantoni's culture experiments did not exhibit luminescence in the culture media. Buchner remarks in this connection that the sceptic finds, in this circumstance, grounds for interpreting the cultured bacteria as contaminations. He also points out that a doubter might be inclined to call these bacteria "plastids," "vacuoloids," or "mitochondria." However, Buchner refers to a similar condition of affairs in connection with his own experiments on some *parasitic* luminous bacteria in the wood louse. When these bacteria were cultivated in artificial culture media they did not exhibit luminosity. When the cultured organisms were inoculated into a wood-louse the luminosity returned. Buchner believes that the symbiotic bacteria cultivated by Pierantoni might have responded in like manner.

The cells containing urate crystals do not belong essentially to the luminiferous organ. They are absent in this organ in some species, and are present in the fatty tissues of some insects which are not luminous. It was mentioned above that symbiotic bacteria may be associated with these cells.

There is considerable variation in the tracheal supply to the luminiferous organs in different species. M. Schultze described the morphology of the trachea in the *Lampyridae*. He found the finer tracheal tubules extending into the cells. It has been shown by various authors that oxygen is utilized in the light-producing process in these forms. The experiments of Molisch in this field are conclusive. When air is denied to light-producing bacteria there is no luminosity, but when a bubble of air is brought in contact with the bacteria they again emit light. Beijerinck ('89) mixed a bouillon culture of luminiferous

bacteria with a leaf extract containing chlorophyl. When the mixture was kept in the dark for some time, the bacteria showed no luminosity, but if the mixture was then exposed to sunlight for only a minute the bacteria became luminous. The light from a burning match was sufficient to restore the luminosity of the organisms after they had been kept in the dark for some time. By means of these experiments it was possible to detect very minute quantities of oxygen liberated by the chlorophyl. Molisch utilized the luminiferous bacteria to test for the complete absence of oxygen in anaerobic culture media. Very small amounts of oxygen in the media were sufficient to produce luminosity in the bacteria.

In the beetles, whose luminosity appears to be intermittent, Dubois, Verworn and others, have observed that the light does not entirely disappear between "flashes." Dubois experimented extensively with these beetles, and found that when he checked the flow of blood by bending the prothorax, the "flashes" did not occur. When he pressed on the abdomen and forced the blood along more rapidly, the "flashes" coincided with each compression. The tracheal tubes, apparently, also bring oxygen to the cells containing the luminiferous bacteria. The nature of the mechanism, that is responsible for the "flashes" in insects is not definitely known.

Luminosity in the tunicate, *Pyrosoma*, was observed centuries ago. Buchner, recently, has investigated these forms, and has traced particularly the luminiferous symbionts in the more or less complicated development of the host. Various interpretations have been given by different authors regarding the nature of the luminosity. Buchner has demonstrated cells that contain the light-producing microörganisms which are comparable to the mycetocytes. He believes that the microörganisms are fungi, although he thinks it is quite possible that they are "bac-

teroid" stages of light-producing bacteria that have adapted themselves to an intracellular life. It is not clear from Buchner's discussion why he considers these forms fungi. So far as their morphology is concerned they might as well be bacteria. Although it is immaterial whether they are called fungi or bacteria, they are nevertheless intracellular microsymbionts.

The most remarkable instances of luminiferous organs associated with known microsymbionts occur in certain cephalopods. Pierantoni ('17, '18) and Chun ('10) have described the development of the organs in *Rondeletia minor*, *Sepiola intermedia* and *Pterygioteuthis maculata* (fig. 2). The luminiferous organ in the adult has the general appearance of an eye. It possesses a "tapetum" (dark screen) in the deep peripheral part of the organ. Next to the tapetum nearer the surface is a contractile reflector. In the central part of the organ and superficial to the reflector is a gland-like structure containing the luminiferous bacteria. A lens is present in front of the luminiferous body, and is covered by transparent epithelium. The development of this organ is remarkable in that it utilizes accessory structures in its formation. There are accessory "nidamental glands" found in these animals which later become the luminous element in the light organ. Bacteria are associated with these glands, but they are usually not luminiferous in the earlier stages of development. Modifications of these glands take place, and they come into association with the ink bag, which ultimately forms the tapetum. Between the ink bag and

FIG 2. DIAGRAMMATIC SECTIONS THROUGH THE LIGHT ORGANS OF *Rondeletia minor* (A), *Sepiola intermedia* (B) AND *Pterygiotheuthis maculata* (C)

Ep = epidermis, Le = Lens, Lu = luminous part, P = pigment (in A and B, ink bag) Re = reflector. A and B from Pierantoni, C from Chun (after Buchner).

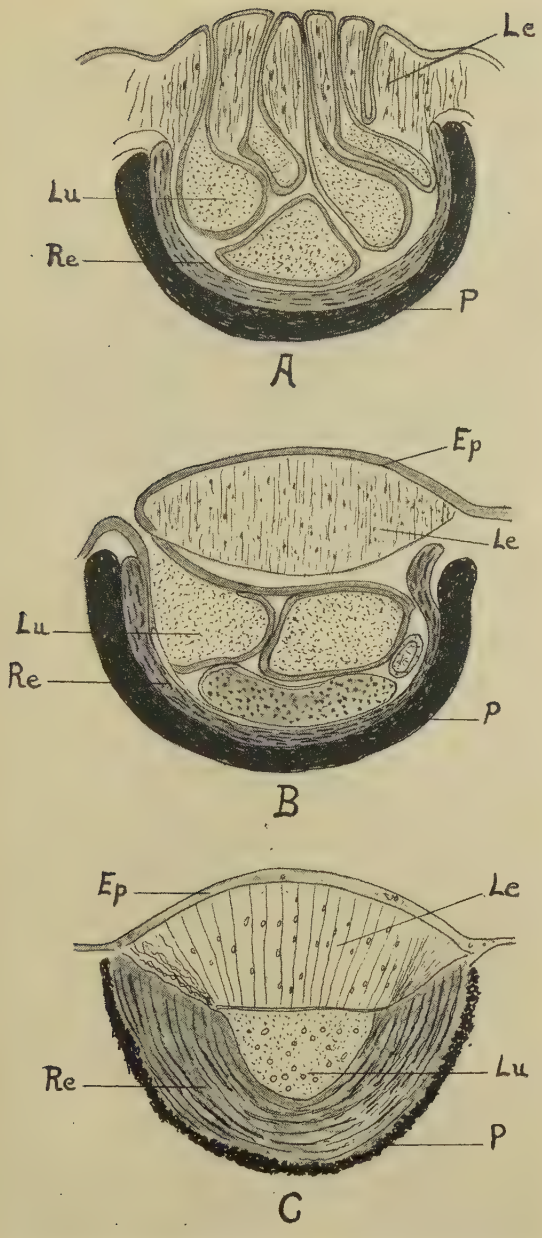


FIG. 2

the accessory "nidamental gland," muscular tissue grows in from the sides and is transformed into the contractile reflector. Superficial to the region of the "nidamental gland," connective tissue grows in, and becomes transformed into the lens. The "nidamental gland" portion contains the luminiferous bacteria, whereas other symbiotic bacteria, which are not luminous, are associated with the formation of the lens. In one species the luminiferous bacteria do not appear in the organ until after it has completely formed. Here is an example of an organ, in which the whole of its complicated structure is essentially a response to bacterial influence, and the entire structure apparently is designed to utilize symbiotic bacteria.

There are other instances of organ formation, associated with symbiotic luminiferous bacteria, but these examples sufficiently illustrate the physiological and morphological responses to such organisms. Harvey ('20) has written a monograph on "The Nature of Animal Light" in which various aspects of "bioluminescence" are considered. This treatise abounds in observations and experiments on the physiology and chemistry of light production. In a number of organisms the light producing materials, luciferin and luciferase are present in the cytoplasm of the luminiferous organ cells, usually in the form of small bodies or granules. The probable genetic origin of this granular material is indicated in the symbiotic relationships that have been discussed and will be considered more fully in a later chapter.

Microsymbiosis is not limited to associations with animal hosts. There are a number of well-known microsymbiotic relationships in plants. The physiologic and morphologic responses in these symbioses are as significant as those in which an animal is the relative symbiont. We shall only discuss at this time one of these symbioses—the root nodules and their symbionts in the legumes.

The presence of bacteria in the root nodules of the legumes was first established by Winogradsky. These symbiotic bacteria (*Bacillus radicicola*) have been the object of intensive research by a host of investigators. It has been demonstrated that they have the faculty of fixing atmospheric nitrogen, and rendering it available for use by the host plant. The microörganism is present in the soil. It enters the root hairs of the host plant and comes to lodge in some of the root hair cells. Its presence stimulates these cells to special growth, and an organ,—the root nodule—is formed. The invading organisms are minute rod-shaped structures. After a sojourn for some time in the root nodule cells, they become transformed into rather large bacilli and branched Y-shaped forms. In the older cells of a nodule, the bacteria have become still more modified. In sections of the nodule stained by a mitochondrial method, the author found large globular forms in the oldest part of the nodule (Wallin, '22b). Löhnis ('21) had previously observed these forms, and believed that they represent "regeneration bodies," but the true significance of these globular forms is not known. In the young root nodules no other organisms but the *Bacillus radicicola* are present in the cells. As the nodules mature, other microorganisms begin to invade the cells of the nodule. In an old nodule, very few if any *Bacillus radicicola* can be found in the nodule. They appear to have been replaced by a variety of parasitic microörganisms.

In recent years botanists and bacteriologists have demonstrated the presence of nodules containing bacteria on the roots of a number of plants other than the legumes. The physiological significance of these symbiotic relationships is not known in most instances. There are also a number of plants that contain extracellular symbiotic bacteria in the leaves and other parts of the plant. *Psychotria bacteriophila* is an example of such a plant in which it has been

shown that the microsymbionts are transmitted in the seeds from one generation to another.

The examples given above, while far from exhausting the list, suffice to illustrate the prevalence of microsymbiosis in nature. It is obvious that a phenomenon which is so common must be of some fundamental significance, and can not be dismissed as a biological digression or mere curiosity. An analysis of the responses of microsymbionts given in the next chapter reveals the fundamental importance of these associations.

CHAPTER VII

An Analysis of Symbiont Reactions

The entrance of a microörganism into the tissues of a plant or animal may result in various types and degrees of response. In many instances, there appears to be no response in either the host or the microörganism. In other cases, the host organism responds in both a physiological and morphologic manner. The resistance of the host may be slight, resulting in "disease," or it may be active and bring about the destruction of the invading microorganism. There may be a morphologic response in the host, resulting in new tissue formation. When such a tissue production is injurious to the host, we may recognize it as a neoplastic disease (tumors, crown gall, etc.). A catalogue of the known responses in plants and animals to microbic invasion would reveal a multitude of types and degrees of relationships and reactions. The responses, in general, may be divided into two types; physiologic and morphologic. Further, these responses may be either temporary or permanent. Our study is particularly concerned with those modifications in physiology and morphology which may become permanent in a species. It becomes necessary also to refer to some of the temporary responses associated with disease in order to understand more fully the factors involved in Symbionticism.

The reactions that have been indicated in the relative or host symbiont in the previous chapter were seen to vary in different symbiotic relationships. In the protozoa, it has not been possible to recognize any response, either physiologic or morphologic. Obviously, if a protozoan animal develops an absolute symbiosis with a bacterial organism,

and there develops a permanent morphological variation in the host, this would constitute a new species, or, at least, a new variety. The determination of the progenitor of the symbiotic variation might then be purely a matter of chance. A physiological response in the protozoan host would be even more difficult to determine specifically. On account of these obvious difficulties, our analysis must be limited, more or less, to the multicellular organisms, in which morphologic variations can not only be traced in ontogenetic development, but may also be recognized in a tissue.

The simplest type of animal that exhibits a definite morphologic response to the presence of a microsymbiont is the *Coelenterata*. In the Portuguese man-of-war, the cells in the canals of the so-called liver that contain algal symbionts are different in appearance from the remaining cells. So, also, in *Adamsia diaphana*, a hexacoral, the cells of the septa that contain the symbionts are morphologically distinct from the remaining septal cells. It might be said that the morphologic variation in the cells preceded the invasion of the symbionts, and that this implies a physiologic variation which produced prototactic conditions that attracted the algae. Such an interpretation, however, would seem to place the "effect before the cause." The special cells are not present before algae make their appearance. A comparison with related, but non-symbiotic species, reveals the fact that the special cells are not present. Buchner has shown that the algal symbionts are carried in special cells in a large number of animals. These special cells develop in conjunction with the algal symbionts, and hence were called "mycetocytes."

Morphologic reactions in the relative symbiont are specifically shown in the turbellarians, *Convoluta roscoffensis* and *C. paradoxa*. In other turbellarian worms, an excretory system elaborates and functions, while in the above-named species, the excretory system fails to develop. The

absence of this system in these worms is associated with the presence of algal symbionts. Again, it might be argued that this failure in development of an excretory system produced prototactic properties in the worms that are specific for certain algae, and that this circumstance is the crucial factor in the development of the symbiotic relationships. This explanation would not supply a cause for the lack of development. Since excretory systems are present in closely related, but non-symbiotic species, and in the absence of any other evident factor, it appears that the symbiotic algae may be responsible for the absence of the excretory system in *Convoluta roscoffensis* and *C. paradoxa*. It is not possible for us to explain the nature of the mechanism whereby the presence of the algae brings about the permanent agenesis of an entire organ system. We can understand how the loss of activity of an organ may result in complete atrophy, but we do not know how such a deficiency can be so impressed upon heredity that the "defect" shall be permanent in succeeding generations. The presence of a foreign microörganism, suggests the possibility that the microsymbiont in some way influences the germplasm of the host. The absence of the symbionts in the eggs of the host, also complicates the situation. True the symbionts are present in the egg capsule which contains the elements of food necessary for the symbiont, but the symbionts do not enter the developing worm until it has reached a certain stage in its life cycle. We must assume that if the microsymbiont impresses a new characteristic, or perhaps, better stated, destroys a potential characteristic in the germ plasm, this must be accomplished while the microsymbionts are present in the general or somatic tissues of the host.

The loss of the property of food-ingestion in *Convoluta roscoffensis*, indicates a definite and fixed physiologic response to the presence of the algal symbionts. This

physiologic modification, however, does not appear to be impressed upon the germ plasm, as the young worms take in food from the out-side like any non-symbiotic turbellarian. This response appears to be limited to a definite growth period of the individual.

The presence of special cells containing bacterial symbionts in the *Blattidii* (cock-roaches) is another example of morphologic response on the part of a host to the presence of microsymbionts. We know nothing of the functional significance of the "bacteriocytes" in these insects. The microsymbionts are transmitted from one generation to another in the germ cell.

In the plant kingdom, the formation of root-nodules in conjunction with the invasion of microörganisms represents a specific illustration of a morphologic response in the host. Wright[1] distinguishes two types of bacteria which produce root nodules on the soy-bean. These two types are morphologically alike, but have different serological and cultural characteristics. The nodules produced by the two types have a different arrangement on the roots of the host plant. In these plants the biologic association of the symbionts has not developed to a point of absolute dependence, although the association apparently is valuable to both.

The symbiotic relationships present in all the lichens represent one of the most significant examples of symbiosis that we shall attempt to analyze. The group of lichens is distinctive in that every member of it is a symbiotic complex. The plant consists of algae symbiotically combined with a fungus. The lichens are particularly significant and instructive since the various species exhibit different degrees of symbiotic relationship. In the simpler forms, reproduction of the two symbionts is independent. It is necessary for the offspring of the two symbionts to find

[1] Personal communication.

each other, and again join in the production of a daughter lichen. In the next higher group, reproduction of the two symbionts is still independent, but an accessory method of multiplication is represented by a process of budding. The daughter lichens produced by this method contain both of the symbionts from the first. In the higher groups of lichens, a reproductive organ, the "soridium" is formed. Both symbionts supply materials in the formation of the reproductive elements. In these higher lichens the symbiosis has become *absolute*, and reproduction is no longer independent. The relationships in these three groups of lichens appear to indicate that there is a gradual development towards a complete interdependence of the two symbionts.

In the more lowly lichens, there is a possibility of *independent existence* of the two symbionts. In the higher lichens, the two symbionts are incapable of independent existence in nature. There is no question regarding symbiosis in the lichens, as the symbionts are readily distinguishable. Here is an example of an entire group of organisms whose origin unquestionably *depended upon the establishment of a symbiotic relationship.*

The symbiotic relationships in which luminiferous bacteria are the definitive symbionts, have produced morphologic responses in the host that are of the greatest significance in the theory of Symbionticism. In a number of these symbioses, definite organs have been formed in the host, associated with luminescence and harboring the luminiferous symbionts. These organs vary in complexity in different animals. In some of the beetles, the organ consists of two kinds of cells—the bacteriocytes, containing the luminferous symbionts, and the "urate cells" which become associated with them and have a subsidiary function in connection with the organ as a whole (reflector). The cells that harbor the luminiferous symbionts are modi-

fied chitin cells, according to the authors who have investigated them. Here is a morphologic response in which not only chitin cells become modified by the microsymbiont but other cells (the "urate cells") are apparently attracted to the bacteriocytes, take up a definite position, and contribute to the formation of the luminous organ. This is a comparatively simple organ, performing a definite function, but its origin is directly traceable to bacterial invasion. The organ is a permanent and characteristic structure of the species. The microsymbiont is transmitted in the egg cell from generation to generation.

The organ associated with light production in the cephalopods (*Rondeletia minor, Sepiola intermedia, Pterygiotheutis maculata*) is rather more complex than the luminiferous organ in the beetles. In these forms we recognize four distinct tissues entering into the formation of the organ. The bacterial symbionts associated with the first structure to develop—the "accessory nidamental glands"—do not at first possess luminescence in some species. It is only after the organ is completely formed that the luminiferous bacteria make their appearance in that part of the organ formed from the "accessory nidamental glands." It is not definitely known if these symbionts are a transformation of the original bacteria associated with the "nidamental glands," or if they come from some other source after the organ is formed. On the basis of experiments with cultured luminiferous bacteria, it is safe to assume that the original bacteria become modified during development and become luminous. One of the accessory structures in the organ (lens) is definitely known to be associated with bacteria in its formation.

The history of the development of the luminiferous organ in the cephalopods illustrates the influence of microörganisms in organ production. Various types of cells, apparently under the influence of the essential symbionts, become

modified and associated in the organ. The behavior of the microsymbiont in this symbiosis is also significant and will be discussed presently.

We have followed some of the responses that take place in the relative symbiont, or the host, in symbiosis, we have next to consider the modifications that arise in the definitive symbiont. These are the more difficult to analyze, but they form the "connecting link" in the theory of Symbionticism. It is in this connection that the responses shown by algal symbionts furnish important evidence.

Keeble and other investigators have described the modifications that algae undergo in the symbiotic situation. In the first place, some of the algal species that enter into symbiosis are flagellate in the free-living condition. These flagella are lost when the algae enter the tissues of the host. Some species, after they have taken up the symbiotic position undergo further modifications; the cell wall, nucleus, and pyrenoid disappear, so that all that remain of the original algal cell are the chloroplasts. These chloroplasts, apparently, continue to perform their normal functions, for starch granules can be demonstrated by the iodine reaction in the cytoplasm of the host cell.

We may then inquire,—what is the nature of the chloroplast? It has been looked upon as an "organ" in the plant cell. Just what is meant by an intracellular "organ" is difficult to determine from its usage in the literature. In general, it appears that the conception of cytoplasmic "organ" has carried with it the idea of a passive or non-living body. Such a conception is difficult to correlate with what we know of the activities of chloroplasts. We might further ask, Is the chloroplast only a "container" for the chlorophyl, and is the chlorophyl only a sort of "catalyzer?" We can extract chlorophyl from plants, mix it with carbon-dioxide and water and place the mixture in sunlight, but no starch is produced. There appears

to be some sort of an accompanying organization necessary if chlorophyl is to produce starch. This "organization," apparently, is present in the chloroplast. What, then, is the nature of this "organization?" Is the chloroplast a *living* body? We do not know, but we have good reasons to suppose that it *is* a living body. This will be discussed in the following chapter.

How are bacteria modified in symbiosis? The answer to this question is complicated by a multitude of variations in the responses of different strains of bacteria. Some of these can hardly be more than guessed at in our present state of knowledge. When a bacterium is subjected to a cytoplasmic environment, a number of factors become operative—physical, chemical, thermal, photic and electrical. We know that free-living bacteria may be influenced by the same set of factors. It is a comparatively simple matter to determine the morphologic responses of bacteria in artificial culture media. We can determine some of the physiological and chemical modifications of microörganisms under these conditions, but corresponding examinations of symbiotic bacteria cannot readily be accomplished. In many instances, bacterial symbionts exhibit pleomorphism, but free-living bacteria may exhibit the same tendency (Reed, '24). The responses shown by some pathogenic bacteria illustrate types of response that may be present among microsymbionts.

Pleomorphism or morphologic variation, although not limited to microsymbionts, is commonly present in symbiosis. This has been observed by a number of investigators, and is especially prominent in *Bacillus radicicola* in the root nodules of legumes. The symbiont at the time of invasion is a minute rod-shaped organism. Later it develops into a much larger organism, and becomes forked at one end. Finally it transforms into a comparatively large, globular body. These transformation stages, apparently, do not occur in the free-living state.

Another property developed by the *Bacillus radicicola* in the symbiotic state, is that of "fragility." This is especially prominent in the final morphogenic state—the large globular body. In an ordinary bacteriological preparation in which the nodule is crushed on a slide, the large bodies are practically never seen, undoubtedly, due to their fragile nature. In histological sections, the large globular form is difficult to demonstrate with the ordinary technique. The organism of Rocky Mountain spotted fever and other Rickettsia bodies exhibit similar fragility. All bacterial symbionts do not necessarily exhibit the same degree of fragility, but on the contrary, many microsymbionts appear to retain their hardier properties.

It is a difficult matter to explain this variation in fragility. In general, it is safe to assume that fragility is a consequence of symbiosis; the greatest degree of fragility is associated with absolute symbiosis. Other factors, however, must be considered in any attempt to explain fragility. It must be recognized that the chemical constitution and the physiologic activity of the symbionts may be different in every symbiotic complex. We must also admit that there may be different "degrees" of symbiosis, as exemplified in the three groups of lichens. When a symbiosis is as "loose" as it is in the lower lichens, the individuals still retain their hardier characteristics, and are readily capable of an independent existence. On the other hand, when the symbiosis is as "close" as it is in the higher lichens, the symbionts have lost their hardier characteristics and are no longer able to maintain an independent existence.

The "degree" of symbiosis determines, in a large measure, the independence of the symbionts. This circumstance has a bearing on the difficulty in cultivating microsymbionts in artificial culture media (see Meyer, '25). In some forms in which the symbiosis is not so close, the microsym-

bionts are readily cultivated. The physiological attributes of the microörganisms also must be taken into account. The microörganisms responsible for a number of diseases can be demonstrated in the tissues and in the blood, but attempts at artificial cultivation of the assumed causative organism have only resulted in failure. Obviously, if the proper ingredients were supplied in the culture medium, and other crucial factors were properly adjusted, the microörganism in question should grow freely in this situation. The Rickettsia bodies of Rocky Mountain spotted fever have resisted cultivation in artificial culture media in the experiments of Wolbach, Noguchi, and many other investigators.

Pathogenic microörganisms exhibit properties that throw light on some of the possible responses of microsymbionts. It has been shown by a number of bacteriologists that the "specificity" of certain microörganisms may be altered when grown in certain culture media. It is also a common observation that the viability of some bacterial cultures diminishes with the age of the culture. It has further been established that the morphology of a microörganism may be altered, by changing the environmental factors. Above all, it must be emphasized that our knowledge of the behavior of bacteria is decidedly limited.

Variations in response of microsymbionts is exemplified in the luminiferous bacterial symbionts. Harvey ('20, '24), who has especially studied the physiology of bioluminescence, states that Zirpolo ('17, '22) cultivated bacteria from the luminiferous organ of *Rondeletia minor* (a squid), while Montora ('22) was unable to grow bacteria from a similar organ in another squid, *Hetoroteuthis dispar*. Attempts to cultivate bacteria from the luminiferous organs in a number of other animals have resulted in failure. It is perhaps this circumstance, in part, that led Harvey to say. "I believe the animals in which luminous symbiotic bacteria can be demonstrated are relatively few."

The experiments described in a previous chapter, demonstrated a variation in the fragility of mitochondria at various periods in the life of the animal. It was shown that they are hardy in the fetus and new born, and gradually develop fragile properties with the age of the animal. It was, also, indicated in these experiments that the mitochondria of the adult liver tissue do not grow readily in a medium which supports luxuriant growth when the mitochondria are derived from the liver of the fetus and the new-born. These results indicate the danger of arriving at positive conclusions from negative results in bacterial culture experiments.

Another observation in connection with the physical nature of mitochondria is significant in connection with the ultimate morphologic response of a microsymbiont. Cowdry and other investigators have described cells in which the mitochondria appeared to have gone into "solution" in the cytoplasm. The author has also observed and described the same phenomenon in lymphocytes. It was supposed that some injury or accident was responsible for this condition. In the light of the response shown by some algal symbionts in which the cell wall, nucleus and pyrenoid disappeared in symbiosis, it now appears that another interpretation is possible for this mitochondrial "solution" phenomenon. It is possible that the so-called "dissolution" is a normal end-response of microsymbiosis, or may represent a temporary condition due to variations in surface tension either in the host cell or the microsymbiont. In the latter event, it might be possible for the mitochondrial material to separate again and reform into bodies when the surface tension returns to its original condition. Biologists are unanimous in the view that protoplasm is a colloidal complex. It differs from an artificial colloidal mixture in so far as it contains what we call *life*. Does this additional property (life) modify the

nature of protoplasm so that it does not respond to the influence of chemico-physical forces? There is no evidence at hand to show that such is the case. On the contrary, it is well known that many responses of protoplasm are due to chemical and physical influences. Obviously, it will require more extended observations and experimental analyses to determine the nature of these reactions in mycrosymbiosis.

CHAPTER VIII

Symbionticism and the Origin of Species

When a variation develops in a living organism and this deviation becomes hereditary, we recognize a new species or a "new variety," depending upon the extent of the variation. In the chapters on "Microsymbiosis" and "An Analysis of Symbiont Reactions," a number of examples were given in which tissues and organs were shown to originate as the result of the invasion of microörganisms, or Symbionticism. The variations that were present in these forms were of sufficient magnitude to constitute new species. In the majority of these examples, it was possible to recognize the essential cause of these variations by the particularly prominent characteristics of the microsymbionts concerned. Various biological conditions and phenomena, coupled with the bacterial nature of mitochondria and the responses of organisms to microbic invasions, leads the author to conclude that *Symbionticism is the fundamental factor in the origin of species.* While the above mentioned evidence furnishes the basis for the hypothesis, it is pertinent to test the theory further, and attempt to determine how it articulates with other known biological phenomena.

If bacteria are the building stones, or the "primordial stuff," from which all higher organisms have been constructed and modified, then we should be able to find in bacteria the same metabolic products that are found in the specialized cells of the higher forms of life. The researches that have been done on bacteria are so extensive that it is beyond the realm of possibility for any one investigator to acquaint himself with the entire literature. The

summaries and data which may be found in text-books and monographic works on bacteriology, however, contain many examples of known bacterial activity which form valuable evidence for our purpose. It has been claimed that there are no enzymatic properties in the human body that are not also represented in bacteria (Jordan, '24). It may not be possible to completely verify this statement. Evidence that supports this however is abundant. In Marshall's "Microbiology" ('21) considerable attention is given to the metabolic products of microörganisms. After a perusal of this book, it is evident that bacteria produce metabolic products that are identical in their properties with those elaborated by plant and animal cells. A brief summary of some of these activities may serve to illustrate this point.

The better known enzymes of animals and plants are concerned with food digestion. While we do not know the chemical composition or structure of any of these enzymes, their chemistry is known in terms of the reactions they produce with carbohydrates, fats, and proteins. These enzymes are known to be the products of various glands collectively known as "digestive glands" in the higher animals. Some of them are also present in plants. The enzyme concerned with starch decomposition—amylase (ptyalin, diastase)—is a particular product of the salivary glands, and also of the pancreas. Amylase hydrolyzes starch and changes it into maltose, a sugar. Regarding the occurrence of amylase in nature, Marshall states: "Diastase, or amylase is a starch-dissolving enzyme which is one of the most common enzymes in nature. It is found in all green plants, and it forms during the sprouting of starchy seeds. Many moulds and a few bacteria produce this enzyme, while yeasts generally cannot decompose starch for lack of diastase." Cellulose is closely related to starch, and is decomposed into a soluble sugar by an enzyme

called "cellulase" or "cytase." It is interesting and significant in this connection that those animals (the herbivora) in which cellulose forms the chief article of diet do not possess any cytase-producing glands, *but depend upon certain bacteria present in their intestines to supply the enzyme for cellulose digestion.*

Lipase is an enzyme which has the ability to split fat into glycerin and fatty acid. It is produced in the stomach and pancreas of the higher animals and is present in some plants. A few bacteria are known that have the ability to split fat into the same components.

Pepsin and trypsin (proteases) are proteolytic enzymes produced in the stomach and intestines of the higher animals. These enzymes have been little studied in the microorganisms. Marshall states, however, that "there is so far as can be determined, little appreciable difference between the proteolytic enzymes obtained from different organisms, whether low or high in the plant or animal world, consequently many experiences with animal pepsin and trypsin can be applied to microbial enzymes."

The blood-clotting enzyme, thrombase, does not occur in microörganisms, according to Marshall.

Rennet is a substance present in the stomach of calves. Rennet is found in many species of microörganisms. Rennet-forming bacteria are found in milk and dairy products, in soil and other habitats.

Pigment formation is an activity that is widely distributed in plants and animals. Many bacteria are known in which pigments are produced.

A few strains of bacteria are known that have the property of decomposing hemoglobin. One of these forms is *Streptococcus hemolyticus.* Certain functions in the mammalian body are of a similar nature. It is believed that the spleen has the property of destroying red blood corpuscles when their hemoglobin content falls to a certain

level (Hemmeter, '26). The liver is known to have the property of decomposing hemoglobin.

The suprarenal gland, apparently, has the property of producing adrenalin or epinephrin. Mathews ('15) states that it has been claimed that certain bacteria have the property of changing tyrosin into adrenalin.

From this brief survey it is evident that many of the products that are characteristic of certain specialized cells in higher animals are also the products of bacterial metabolism. The basis for a correlation of this circumstance is to be found in the bacterial nature of mitochondria. Symbionticism offers a ready explanation for the origin of the "specialized cells" in the higher plants and animals. The probability that Symbionticism is the factor concerned in the acquisition of these specialized properties, is enhanced by the results of a number of mitochondria investigators. Regaud and Mawas ('09) concluded that the secretions of the parotid and submaxillary glands originate from the mitochondria. Prenant ('13) associated pigment formation with mitochondrial activity; Mulon ('10) concluded that the secretion of the suprarenal glands originates from the mitochondria; Hoven ('10), Horning ('25) and others believe that zymogen of the pancreas originates from mitochondria. Other cell products and structures that have apparently originated from mitochondria are catalogued in Chapters II and IV.

Modern researches in the plant kingdom have revealed some significant developmental activities. Our interest is especially directed to the chloroplasts and other plastids. Practically all green plants—the *Bryophyta, Pteridophyta* and *Spermatophyta,* as well as the lowly algae—possess chloroplasts which in turn contain chlorophyl. The *Cyanophyceae* (*Schizophyceae*), or blue-green algae contain no chloroplasts. In these forms, the chlorophyl and the blue-pigment (not always blue, the characteristic hue of the

Red sea is due to *Cyanophyceae*) are diffused throughout the cytoplasm. The *Cyanophyceae* do not contain a *distinct* nucleus. In the central part of the cell, there is a material that has nuclear staining properties and is usually considered a nucleus, but it lacks a nuclear membrane in most cases. The *Cyanophyceae* together with the bacteria (*Schizomycetes*) constitute a plant division, the *Schizophyta*, in the opinion of most botanists. In other words, the blue-green algae are similar and closely related to the bacteria.

The origin of the chloroplasts in algae and higher plants has interested botanists for many years. Schimper ('85) first suggested the continuity of chloroplasts in development. He believed that chloroplasts arise from preëxisting chloroplasts by a process of division. Guilliermond ('21) and others have shown that chloroplasts originate from mitochondria in ontogeny by a process of transformation. The various stages of transformation have been so clearly illustrated by Guilliermond that very little doubt exists in regards to the accuracy of his observations. His discoveries have been confirmed by a large number of investigators, and this method of plast-formation has been introduced into text books. Not only are chloroplasts formed in this manner, but other kinds of plasts (chromoplasts, amyloplastids, leucoplasts) are also formed from mitochondria by a process of differentiation. The chlorophyl in these plastids is not apparent in the mitochondrial stage of development, but appears in later differentiation. In many instances a pyrenoid is associated with the chloroplast. The shape of the plast is somewhat variable. It is usually circular in outline, but in *Euglena viridis* (a protozoan) it is star-shaped, while in *Microglena punctifera* (also a protozoan) it is in the form of two elongated rods.

It is evident from the data that have just been given, that chloroplasts and other plasts of the higher green plants

bear a close morphologic similarity to the blue-green algae (*Cyanophyceae*). Physiologically the two structures are also similar. The possible phylogenetic origin of plasts from the blue-green algae appears to be a logical conclusion in line with Symbionticism. How are we to harmonize the mitochondrial origin of plasts in ontogeny with a relationship to the blue-green algae? Are the plasts to be considered transformations of symbiotic bacteria? It must be mentioned in this connection, that a large number of bacteriologists claim that no bacteria are known which contain chlorophyl; other bacteriologists, on the other hand, insist that chlorophyl is present in some forms. A number of circumstances and experiences, however, assist us in correlating and harmonizing the plasts with Symbionticism. It has been shown in microsymbiosis, and particularly in the behavior of mitochondria, that microsymbionts may be altered by 'their environment. The mitochondrial pleomorphism experiments clearly demonstrated the pleomorphic responses of these microörganisms to slight variations in the environment. The absence of chlorophyl in the mitochondrial stage of the chloroplast is not a barrier to the acceptance of a genetic origin from the blue-green algae. It was shown in connection with the development of the luminous organ in some squids, that luminous bacteria were not present until the organ was completely formed. Extrinsic factors as well as inherent tendencies operate in the development of "specificity" in microörganisms. It has been shown also by various investigators, and is commonly accepted that light is a fundamental factor in the production of chlorophyl.

These observations and experimental results certainly indicate reactions and processes in microörganisms, particularly microsymbionts, that clear the way for a better appreciation of the possible genetic relationship of chloroplasts and the blue-green algae. It may appear that the

immediate mitochondrial origin of chloroplasts is opposed to the acceptance of a genetic relationship to the blue-green algae. On further reflection and analysis, however, such a presumption is hardly valid. The chloroplasts have in the main the characteristics of living bodies. Cell histogenesis in ontogenetic development demonstrates the possibilities of differentiation. It is well known that the germ cell contains potentialities for the elaboration of all the tissues of an individual, whether it be a man or a worm. These "potentialities" are not sufficient *within themselves* to produce the completed individual. It recently has been emphasized by Child ('24) and others, that environmental factors play an important rôle in histogenetic differentiation of tissues and organ formation. These environmental factors, perhaps, are chiefly of a chemical nature, although mechanical and physical factors are known to be involved. The metabolic products of one organ or tissue may be the stimulating influence that inaugurates differentiation in other organs or tissues. According to Stockard ('21), the active differentiation of a tissue or the formation of an organ may exercise an inhibitory influence on the differentiation of a number of other organs and tissues.

Conklin ('19) has given a clear exposition of the status of histogenetic development in the following words: "to a certain extent differentiations are already present in the germ cells, but during the course of development their number is greatly increased. This increase in differentiation is caused by modification of previously existing differentiations, by transformation rather than by formation *de novo*, by evolution rather than by creation. New things appear in the course of ontogeny because of new combinations of things already present, just as new chemical substances with new qualities appear as a result of new combinations of elements."

The genetic origin of chloroplasts and other plasts from

the blue-green algae presupposes a morphologic reaction, upon the acquisition of the symbiotic relationship. This is in harmony with the usual reactions observed in microsymbionts. Furthermore, it harmonizes with the adaptive behavior of living organisms in general.

Merejkovsky ('20) has recently advanced the hypothesis that the chloroplast is a microsymbiont, genetically related to the blue-green algae, and that all the higher green plants are symbiotic complexes. He has emphasized the morphologic and physiologic similarity of chloroplasts to the *Cyanophyceae*. It appears, from an analysis of Merejkovsky's treatise, that he could not harmonize a mitochondrial origin of plastids in plant ontogeny with a genetic origin from the *Cyanophyceae*. He does not believe that the plastids originate from mitochondria in the ontogenetic development of the plant. In lowly forms like the algae, it is possible for the plastids to divide and multiply as such; the transmission of microsymbionts in algae from one generation to another is a comparatively simple matter. In the higher plants reproduction is complicated, and the transmission of an absolute symbiont must be associated with the germ cell or its accessory parts. The potentialities of the entire plant are crowded into the germ cell; it is not surprising therefore to find the symbiont also modified for transmission. While we agree with Merejkovsky's conception of the symbiotic nature of all the green plants (we would extend his conception to include *all* plants), we cannot agree with his rejection of the association of mitochondria in ontogenetic chloroplast formation. The careful researches of Guilliermond and others, appear to have definitely established this relationship. Merejkovsky, perhaps has been misled by the assumed fixed phospholipin constitution of mitochondria.

The question may arise,—Under what circumstances does Symbionticism take place? It is impossible to give a

specific explanation that would fully answer the question. Nevertheless, we can appreciate the complexity of the situation when we review the behavior of known microorganisms and the factors regulating their activities. The reactions of pathogenic microörganisms, perhaps, have been investigated more fully than those of any other group; their behavior is more significant, also, because of the relationship they bear to other forms of life. A brief discussion will suffice to indicate some of the "peculiarities" of microbial behavior, as well as the importance of variations in the host with respect to infection.

A large number of pathogenic microörganisms have a limited specificity as regards the plant or animal species in which they are capable of producing disease. The avian tubercle bacillus, for example, is capable of producing tuberculosis in birds, cattle and swine, but apparently not in man. The bovine strain of the tubercle bacillus produces disease in cattle, man and certain laboratory animals, but not in the horse. Some microörganisms have a marked tendency to localize in certain tissues, for example the globoid bodies of anterior poliomyelitis. The diphtheria bacillus is limited in its infections to the mucous membranes, especially those of the respiratory tract, and to wounds exposed to the air.

The host also plays an important rôle in the determination of infection. The tubercle bacillus does not ordinarily produce definite disease in a normal, healthy individual. When vitality is lowered, the organism may become implanted and develop. The response of the host to an infection, or to the presence of a pathogenic organism varies within wide limits. In a particular type of infection, one individual may respond with a violent reaction while another host may manifest very little or no response. An example of marked tolerance is found in those cases spoken of as "carriers." Typhoid carriers are rather common and may not be conscious of the infection they harbor.

The influence of environmental factors on the response to infection has repeatedly been demonstrated. Pasteur rendered the naturally resistant hen susceptible to anthrax by chilling the bird with cold water. Frogs, which are immune to anthrax at ordinary room temperature, quickly die after an inoculation if placed at a temperature of 25° to 35°C. (Jordan).

Obviously, not all symbiotic relationships result in the origin of species. There are a number of microsymbiotic relationships, apparently, which lack a morphological response. This is especially true in extracellular symbiosis, and probably obtains in some intracellular symbiotic relationships. It is practically impossible to draw a sharp line between parasitic relationships and symbiosis. Furthermore, there may be responses in the host under these conditions that are not permanent.

It is evident that in those cases of symbiosis in which the response in the host is permanent and produces new species, a peculiar combination of factors, both extrinsic and intrinsic, in two distinct symbionts have met and are harmonious. It would be futile to attempt to analyze and determine the nature of these factors. An analysis of fertilization may serve to elucidate their significance. The sperm of a species possess a definite affinity for the ovum of the homologous species. In some instances the sperm may penetrate an ovum of a closely related species and fertilize it. The individual that develops from this fertilization is usually sterile or at least abnormal. Changing the composition of the sea water surrounding the eggs of certain marine animals, may be sufficient to prevent fertilization by the sperm. Certain temperatures may produce similar results. In other words, if normal reproduction is to take place, it is imperative that certain extrinsic and intrinsic conditions be satisfied.

The responses of a host to the presence of a micro-

organism in Symbionticism may not be limited to a single tissue or organ. If the particular microsymbiont has the property of producing some chemical substance, this may not only affect the particular group of cells in the production of a new cell type harboring the symbiont, but the chemical substance may alter the physiology of a number of distant tissues and organs. Such reactions are commonly found in connection with pathogenic microörganisms. The pathogenic organism may be localized in a particular group of cells producing abnormalities in the tissues of that organ, but the toxins may affect specifically other tissues of the host. The "nidamental gland," whose origin is associated with symbiotic bacteria, apparently influences muscular tissue in such a way that it transforms or differentiates into a reflector for the luminiferous organ in the squid. It is possible that many tissues in an animal present secondary modifications or responses to symbiont influences.

It appears to the author that Symbionticism offers a rational explanation for many of the variations in the morphology and physiology of plants and animals. When these variations are of sufficient magnitude and permanence they constitute new species. The fact that mitochondria are universally present in the cells of all organisms higher than the bacteria, that mitochondria are bacterial in nature and that microsymbiosis can determine morphologic and physiologic changes in organs and cells, can lead to no conclusion other than that *Symbionticism is a fundamental causative factor in the origin of species.*

CHAPTER IX

Symbionticism in Relation to Heredity and Development

When Darwin advanced his theory on the origin of species, there was practically nothing known concerning the mechanism of heredity or the factors concerned in development. Darwin felt the need of an hypothesis that would explain the operations of heredity, and advanced his own theory of "pangenesis" to fill this gap. The situation is quite different today; instead of formulating a new theory of heredity to fit our ideas as to the origin of species, it becomes necessary to fit the conception of Symbionticism to a more or less established mechanism of heredity.

Since the rediscovery, in 1900, of Mendel's researches there has grown up an extensive literature on the nature of the factors of heredity. It becomes necessary to limit our discussion to the generalizations and analyses that have been made in reviews on genetics and cytology. We are especially indebted to Wilson's comprehensive treatise: "The Cell in Development and Heredity" ('25); to Morgan, Sturtevant, Muller, and Bridges ('15) for "The Mechanism of Mendelian Heredity;" and to Conklin for an analytic review of the researches in heredity, delivered as a series of lectures under the William Ellery Hale foundation and published in the Scientific Monthly ('19–'20). Space does not permit an extensive review of the data that have been brought forth in these books and papers. We prefer to discuss some of those features of heredity and development that appear to have a direct bearing upon the theory of Symbionticism.

Certain difficulties are encountered in the use of some of

the terminology of the past on account of the more or less specific conceptions associated with these terms. We are immediately confronted by the phrase "acquired characteristics." Although this term is sometimes used with a different meaning from that originally proposed, to many it conveys the idea of modifications that occur in an individual as the result of outside influences of whatever nature. In Symbionticism, it would appear that the acquisition of a microörganism and the morphologic response of the host constituted an "acquired characteristic." The theory of Symbionticism implies that such an acquired character is "impressed" on heredity, and is transmitted from generation to generation. It is apparent, however, that we must differentiate between various kinds of "acquired characteristics." We recognize acquired characters that are the result of extrinsic forces, such as temperature, light, moisture, chemical and physical agents, etc. Other acquired characters are physiologic in nature and may be produced by "use and disuse." We might also include the "acquired characteristics" that are artificially produced, such as dehorning of cattle, cutting of plumage, castration, etc. We agree with the majority of students of heredity, that a mechanism whereby acquired characteristics of this kind might be transmitted in heredity has never been demonstrated nor logically explained. The "acquired characteristics" associated with Symbionticism are of an entirely different nature and relationship to the organism.

It may be necessary to modify some of the ideas that are held in connection with heredity, but these modifications, perhaps, are not so momentous as they might at first appear. It is also proper to point out that some of the conceptions held in heredity are still in the hypothetical stage. Some facts, apparently, have been established in regard to heredity and development and something is known as to the

nature and behavior of mitochondria and microsymbionts. While a few minor points may be at variance, the theory of Symbionticism appears, in general, to harmonize with the modern conception of heredity.

The facts that have been established in connection with mitochondria are few, but to the point. It has been shown by a number of investigators that, in the division of the cell, the mitochondria are more or less equally divided and distributed to the daughter cells (see plate I, fig. 3). Mitochondria are present in the germ cells. In the ovum they usually retain their characteristic appearance while in the sperm they may coalesce to form the nebenkern and spiral filament. Mitochondria increase by simple fission. The evidence for the bacterial nature of mitochondria has been submitted by the author, and reviewed in a previous chapter of this book. Up to the present time, this work has neither been confirmed nor discredited.

The question arises: What rôle do the mitochondria play in the mechanism of heredity? The remarkable researches that have been made by Morgan, Davenport, Castle, Calkins, Guyer, Bridges, Shull, Sturtevant, and many others in this country, deVries, Janssens, Johannsen, Bateson, Doncaster, and others in Europe, have fully established the localization of the heredity transmitting factors in the chromosomes of the nucleus. The history of these researches appear to prove beyond a doubt that the chromosomes are the bearers of heredity. Morgan and his associates, in particular, have been able to correlate certain characteristics with certain chromosomes. The discovery of the odd or X-chromosome by McGlung ('02) made it possible to correlate definite characters with this X-chromosome. Factors that are located in chromosomes of this nature have been called sex-linked by Morgan. By studying variations in the animal or plant, and correlating these with the behavior of chromosomes, it has

been found that "elements" of one chromosome may cross over to another. These "elements" have come to be generally known by the name "genes."

Various modifications in chromosomes have been observed by different investigators. It is only the more extreme variations, however, that can be detected. It is generally admitted that these modifications are abnormal in nature; they result in abnormal distribution of chromosomes in development and are of no importance in evolution. Conklin says:

> Abnormalities in the distribution of chromosomes of a cell persist in all its daughter cells and are not usually corrected. On the other hand, abnormalities in the distribution of cytoplasmic substances to the cleavage cells frequently occur and usually the cell returns to a normal condition by a process of regulation. But when once the normal number of chromosomes has been changed there is no regular means of restoring that number. Momentary changes in the temperature, density or chemical composition of the medium may thus produce permanent changes in the distribution of chromosomes and of germplasm. Such changes in the distribution and number of chromosomes are usually of so gross a character that the development is not only very abnormal but also the resulting organism is incapable of continued life and reproduction. Consequently such changes do not usually give rise to new races or species; only in the most favorable cases, where at least the full set of haploid chromosomes is present does the new form survive and reproduce.

The central thought that has developed in connection with the researches in genetics is focused on "genes." The investigations that have led up to this position are too numerous to review here. To those who may doubt the validity of this concept, we can only refer to the extensive reviews and discussions which have been published—such as Conklin's, Morgan's, Bateson's, Wilson's and others. Certainly, the experimental and observational evidence

points to a smaller unit than the chromosome, whatever the nature of this unit may be. The genes have been thought of in terms of atoms or molecules. The work accomplished in genetics in which genes have been accepted as the transmitting unit, has enabled biologists to appreciate the nature of the mechanism concerned in *hereditary variations*. But we must raise the question: Do *hereditary* variations lead to *evolutionary* variations?

The variations that have been experimentally produced by deVries and others, apparently, are *hereditary* variations, explainable on the basis of the behavior of genes. But have these mutations represented true species? Many modern biologists are not convinced that they are stable species. Such mutations may breed true for a time, but it appears that these variations are not of the character nor of the rank of evolutionary variations. We must attempt to look further into the hypothetical nature of genes, and attempt to correlate "genes" with evolutionary variations.

It appears from a perusal of the literature that "genes" are to be considered as some sort of molecular entities, *of the same nature in all forms of life, but varying in their arrangement and grouping in the chromosomes of different species*. This conception, apparently, carries with it the idea that *new genes* are never added to the chromosomes, but that the genes in an amoeba are the same as those in man; the essential difference being the arrangement and grouping in the chromosomes. It is believed also that the genes may be modified by intrinsic environmental factors. Morgan, perhaps, had this conception of genes in mind when he said, "Evolution consists largely in introducing new factors that influence characters already present."

This conception of "preformed" or limited genes has led to more or less bizarre hypotheses on the mechanism of organic evolution.

The "presence or absence" hypothesis assumed that dominant characters are due to the presence of a factor and recessive characters to its absence, and since regressive mutations, in which some dominant character becomes recessive, are much more numerous than progressive ones, it was suggested by Shull, Bateson, and Davenport that evolution might be due to the loss or disintegration of factors or genes. "This conception results," said Shull ('07), "in an interesting paradox, namely the production of a new character by the loss of an old unit," and he suggests that at least the later stages of evolution may be a process of analysis due to the disappearance of one unit after another. Bateson ('14) also proposed the same thing in his well-known inquiry "whether the course of evolution can at all reasonably be represented as an unpacking of an original complex, which contained within itself the whole range of diversity which living things present;" and in the same category is the speculation by Davenport that "the foundations of the organic world were laid when a tremendously complex molecule capable of splitting up into a vast number of simpler molecules, was evolved." [Quoted from Conklin.]

These conceptions, perhaps, have not been generally accepted, although the essential idea of "preformed" or limited genes appears to be retained by most students in genetics.

It is necessary for us to question the logical probability of one feature of these conceptions of genes. Obviously, it is impossible morphologically to examine a gene, but we are justified in entering into a *speculative analysis* of these factors, since all the analyses that have gone before have been speculative. This does not necessarily imply that they have been without a basis. But, just as the most generally-accepted view of the nature of mitochondria was based on an assumed or misleading conception of the chemical constitution of these bodies, so also, it appears that an assumed or misleading conception of the nature of genes has led to a "blind pocket" in heredity and evolu-

tion studies. What evidence is there to assume that *new* genes are not acquired in evolution? The basis for this assumption apparently, has rested on the conception that the "mechanism of heredity is the mechanism of evolution."

Our conceptions in any field of intellectual and scientific endeavor are based upon the analysis of conditions *so far as they have been made.* When more phenomena are discovered, new points of view enter into our speculative analyses, and it may become necessary to alter our previous conceptions. It is evident that the modern conceptions of genes were justified on the evidence that had accumulated. There was no evidence at hand to indicate that "new genes" were acquired in phylogenetic development. A mechanism whereby new genes might be acquired had not been indicated by cytological researches. Only one alternative remained, and that was to assume that life originated as a "tremendously complex molecule, capable of splitting up into a vast number of simpler molecules," or in other words, that the amoeba contains the same genes that are present in man. On the basis of the new point of view that is associated with Symbionticism, we are forced to the conclusion that *new genes must be acquired in organic evolution.* This conception does not alter our ideas as to the behavior of genes in heredity, but it introduces a "new" factor in the conception of organic evolution. This new point of view, furthermore, displaces hypotheses that are antagonistic to the general and broader principle embodied in the theory of organic evolution, namely, that evolution consists of ever-increasing accretions resulting in greater complexity of structure as well as of function. It is logical to assume that there have been ever-increasing additions to organisms during organic evolution. These "additions" must have come from the *outside,* and represent true accretions which are responsible for the origin of species.

The established mechanism of heredity permits the development of variations in organisms, but these variations, apparently do not lead to the production of new species. These variations may be of a retrogressive nature, resulting in the loss of parts or characters, but apparently no *fundamentally new* character is produced by a rearrangement or loss of genes. It apparently is necessary that "new" genes be added to chromosomes in order to produce a fundamentally new character.

How, then, does Symbionticism affect the accepted mechanism of heredity and correlate it with our ideas of organic evolution? The conception that mitochondria may be involved in the transmission of hereditary characters was enunciated by Meves in 1908. A few other investigators accepted this point of view, but the advances in genetics and cytology, and particularly the introduction of the idea of genes, made this position apparently untenable, so that little or nothing is heard of this hypothesis today. It is interesting in this connection to note that certain activities are localized in the cytoplasm of egg cells of some species of animals before the first cleavage takes place. "Localizing activities thus made visible have been studied by many observers, prominent among them Driesch, Boveri, Lillie and Conklin" (Wilson, '25). Conklin ('05) demonstrated three strata in the egg of *Styela partita* immediately following the entrance of the sperm. From the three strata—an upper clear, a lower gray and a middle yellow—ectoblast, entoblast and mesoblast are formed, respectively. Duesberg investigated this problem in *Ciona*, and was able to show a variation in the amount of mitochondria in the three strata. It is difficult from the amount of work that has been done, to determine what the significance of these phenomena may be. Obviously, the variation in the amount of mitochondria in the three regions of the developing ovum signifies something. It does not

necessarily imply that the chromatin of the nucleus has no effect upon the differentiation of the three localized areas.

The acquisition of a new symbiont in the cells of an organism may be the means by which new genes are added to the multitude of those already present in the germ cell. It appears that the more logical and reasonable nature of such a contribution lies in the manner by which a transfer of chromatin material takes place from the bacterial symbiont to the germplasm of the host. When such a transfer is once made, the "new" genes enter into the mechanism of heredity, and constitute new factors which enter into the complex of hereditary variations. It is not beyond the realm of possibility that such new genes not only control their own hereditary characters, but they may modify other characters as well. The possible accretions of chromatin material in Symbionticism will be discussed more fully in the next chapter.

It is well known that mitochondria are prominent in the tissues of the embyro. What rôle do these bacterial bodies play in ontogenetic development? It has been observed by a number of investigators that mitochondria increase by simple fission. It has also been found that mitochondria are more or less equally distributed to the daughter cells in mitosis. As development proceeds the cells become differentiated into specialized groups. What, we may ask, are the factors concerned in this differentiation? In another place, we have mentioned the influence of intrinsic environment acting in conjunction with the hereditary potentialities of the cell. Under the caption of "The Immediate Causes of Differentiation," Wilson ('25) has given a brief account of the ideas that have been advanced on the subject. He says:

> The purely speculative side of this question need not long detain us. DeVries, in his remarkable work Intracellular Pangenesis (1889) considered differentiation to result from the ac-

tivities of invisible, organized, "pangens" (analogous in some respects to Darwin's "gemmules") which migrate from the nucleus into the cytosome and in large measure build up the active cytoplasmic substance. A view similar in all its essentials was subsequently adopted by Weismann, O. Hertwig and other writers. More accessible to investigation are hypotheses which assume differentiation to be effected by the transformation of visible cytoplasmic granules or other definite bodies, arising either by migration from the nucleus or independently in the cytosome; and these views are based to some extent on direct cytological observations. The questions that here arise (p. 720) evidently apply alike to the ovum before its cleavage begins, to the early blastomeres and the embryonic cells derived from them, and to the tissue-cells in so far as they may be capable of further differentiation (by differentiation, redifferentiation and the like).

Existing knowledge of this subject is still too fragmentary and discordant to offer a sufficient basis for adequate discussion. At one extreme are authors who have ascribed cytoplasmic differentiations to the activities of chromidia, extruded from the nucleus of the egg. This view remains to say the least, very doubtful, though there is a certain amount of evidence that certain of the formed cell-components may arise from extruded nucleolar fragments. At the opposite extreme is the view of Altmann, Benda and Meves that the leading rôle in differentiation is played by granules (in particular mitochondria and other forms of chondriosomes) that are of purely cytoplasmic origin, and which possibly may arise by the growth or division of preëxisting bodies of the same kind.

An intermediate position between these opposite extremes is taken by Schaxel in an interesting series of studies on the development of coelenterates, echinoderms and annelids. This observer accepts up to a certain point the conclusions of both sides, recognizing the existence of both chromidia and chondriosomes in the egg and the tissue-cells, and ascribing to both an important rôle in differentiation. Schaxel admits that the chondriosomes or "plastosomes" may become directly transformed into differentiated components of the tissue-cells but

considers the main rôle to be played by extruded nuclear material in the form of chromidia.

This process, according to Schaxel, takes place either at an early period of the ovarian egg (fig. 344) or in the blastomeres or their products immediately preceding the conversion of these cells into the differentiated tissue-cells; and the latter process is carefully described by Schaxel in the mesenchyme-cells of annelids and other objects. Development thus falls into two well-marked periods, an earlier one in which the general framework of development is established in the egg, and a later one in which more specific differentiations are initiated. Schaxel does not, however, believe that the chromidia are directly transformed into the formed elements of the cytoplasm. They disappear as such, having accomplished the initiation of differentiation and localization. The nucleus, therefore, initiated differentiation, while the cytoplasmic elements (chondriosomes and others) are the more immediate agents of the process.

One would like to accept this conception of development and differentiation, which offers so simple a view of the mechanism of the process. Unfortunately evidence concerning the extrusion of chromidia from the nucleus in the manner described by Schaxel is still too conflicting to be accepted without much further inquiry; and the same must be said concerning the direct origin of formed cytoplasmic elements from extruded fragments of nucleoli. On the other hand, it is certain that a large amount of nuclear material, both liquid and formed, is given off from the germinal vesicle in the prophases of the polar mitoses, and to a less extent in the prophases of other mitoses. Experiment has shown that the material thus set free has a most important physiological effect upon the cytoplasm of the egg; and there is evidence that the escaped material or "residual substance" of the germinal vesicle may contribute directly to the formative material of the egg. In the case of pulmonate gasteropods, for instance, Conklin ('03, '10) has shown that the residual material spreads out over the upper hemisphere of the egg and constitutes in large part the upper stratum from which the ectoblast in these animals takes it origin; and he has found reason for a similar conclusion in the ascidian ('05). F. R. Lillie ('06) has reached a similar result

in case of the annelid *Choetopterus*, in which the residual substance is more specifically described as constituted of fine granules or microsomes visible in life before and after their discharge from the germinal vesicle.

Regarding the rôle played by chromidia in cellular differentiation, we must admit that we know very little, if anything. They have been described by a large number of cytologists and have been variously interpreted. The relationship of mitochondria to cellular differentiation assumes a new significance when we bear in mind their bacterial nature and their relationship to Symbionticism. We have mentioned in another place that bacteria in nature are known to produce the same enzymes and metabolic products that are formed by the tissues of plants and animals. Bacteria have been shown to develop intimate symbiotic relationships with plants and animals. In these symbioses it has been observed that "new" cells develop (modifications of preëxisting cells) to harbor the symbionts. Examples have been given in which new organs developed in response to the presence of microsymbionts. Are these observations to be interpreted as biological digressions without any fundamental significance? Must we discard these apparent facts without attempting to correlate them with other cytological observations? In the result of the researches of a large number of investigators, the ontogenetic differentiations of cells have been associated with mitochondria. These researches have not been accepted by most cytologists, apparently because of an *assumed* incompatible chemical constitution of mitochondria. We are forced to the conclusion that *mitochondria constitute a fundamental factor in cellular differentiation.*

If mitochondria, as we have claimed, are the "specific" part of the cell, that is, the part that is concerned with the special function of a differentiated cell, then in cellular differentiation it must be the mitochondria that are es-

pecially differentiated. Certainly, we have a differentiation of the cell as a whole, but this differentiation goes hand in hand with mitochondrial differentiation, and must be more or less dependent upon it. The *evolutionary* origin of the specialized cell depended upon the microsymbiont. The *herditary* or *ontogenetic* origin of the specialized cell must be dependent upon the presence of the same factor (mitochondria). The question then resolves itself into the determination of the nature of the modifications which the microsymbiont undergoes in absolute symbiosis.

We have very little direct evidence on which we can formulate an hypothesis as to the intimate nature of the relationships of microsymbionts to the host cell and to the germplasm. Certain general features of bacteria on the one hand, and mitochondria on the other, are suggestive, and at least furnish a tentative or working hypotheses.

While all bacteria do not contain granules which might be interpreted as chromatin, a great many do. It is a rather singular coincidence that mitochondria have never been observed to contain any granules that could be interpreted as chromatin. One might expect that at least some of the microsymbionts originally (in the free state) contained visible chromatin. We mentioned above the possibility that chromatin material from the microsymbionts might supply "new" genes to the chromatin of the germplasm. It appears possible for a part or all of the chromatin of a microsymbiont to be given up to the nucleus of the host cell and germplasm when symbiosis develops, leaving the remains of the microsymbiont in the cytoplasm somewhat in the nature of a plast. The part of the chromatin removed from the microsymbiont may not be essential for its growth and reproduction, but it is conceivable that the specific activity of the mitochondria is maintained only under the influence of this chromatin. This influence may be exerted 'at long range' from the nucleus. It

may be that this substantiates Schaxel's suggestion that the "chromidia" from the nucleus may function in some manner in connection with the mitochondria in histogenesis.

A certain amount of experimental evidence indicates that the mitochondria undergo modifications during the life of the host. This was suggested by the fragility experiments, as well as by the growth reactions of cultured mitochondria. While these experiments did not demonstrate any variations in the chromatin content (chromatin granules were not visible in any cultured mitochondria), they suggest an alteration in function accompanying a modification in metabolism, as indicated by a change in food requirements. These results were thought to indicate that the mitochondria in the embryo and fetus possess no specific functions, but that these develop or differentiate later in the life of the organism. Obviously, specific tissue and organ function varies greatly in the different tissues of the developing animal. Some functions develop in early fetal life, others certainly not until after birth. Temporary functions (hemopoiesis) are present in the liver before birth and are continued for a short time thereafter.

In a large number of symbioses, the independent properties of the microsymbiont apparently are retained. In some cases, although the microsymbiont may occupy an intracellular position, the bacterial nature of the microsymbiont is readily detected. While we have no way of knowing, it appears reasonable to presume that in such cases of symbioses the bacterial chromatin is retained by the microsymbiont. It also appears possible that in some cases of symbiosis, the microsymbiont completely fuses with the host cell so that it no longer retains a morphologic identity.

The nature of the exact responses of microsymbionts in symbiosis, remains to be determined. One thing appears certain, namely, that if the evolutionary origin of a

specialized cell were dependent upon a microsymbiont, then the differentiation of the specialized cell in ontogenetic development must, primarily, be dependent upon the microsymbionts (mitochondria) associated with that cell.

In a recent paper, Davenport ('25) has attempted to correlate the hereditary variations dependent upon chromosomal behavior with those produced by endocrine disturbances. After directing attention to the hereditary similarities produced by these two methods, he says:

> How can the difference between the views of the geneticist and the chromosomologist, on the one hand, and the endocrinologist, on the other, be reconciled? It would appear that both views can not be true, provided they are mutually exclusive. If the chromosomes are alone responsible for resemblance then the endocrinologists must be deceived in their conclusion. If the hormones are alone responsible for resemblance then there must be something false in the scientific methods of the geneticist. Either of these conclusions is, however, untenable since both the students of chromosomes and of hormones have worked by the best of scientific methods so that their results are unassailable. Hence we must consider both views to be true and that the chromosomes and the hormones each have their rôle to play in the direction of development. The most tenable hypothesis of the nature of the chromosomes is indeed that they are packages of enzymes which activate the metabolic processes of the early stages of development, just as the hormones of the endocrine glands control metabolism in later stages. The hypothesis may be suggested that the chromosomes direct the early stages of development and create certain centers of chemical activity to which they hand over the business of differentiation of particular parts. Thus the chromosomes may work indirectly in establishing certain centers of later chemical activity whose course they have determined, but in the working of whose mechanism they subsequently do not interfere. The endocrine glands of vertebrates represent perhaps a still later and highly specialized stage in the series of regulators of metabolism. In the invertebrates and in plants where such endo-

crine glands are unknown, it is probable that there are, nevertheless, regulating centers constituting various links in the developmental chain which starts with the fertilized egg and its chromosomes and ends with the fully formed parts and tissues. These endocrine glands and other developing controlling tissues are what the chromosomes make them.

This hypothesis receives support from the observations that have been made upon the physiology of the later stages of development, with their processes of folding of membranes, or concrescence, of disruption of parts by mechanical and histolytic processes, and the development of special tissues with their special kinds of form and substance such as are seen in the various connective tissues of the higher animals. These tissues are indeed responsive in turn, not only to the hormones which seem so largely to control their development but also to external agents, such as pressure, gravity and radiant energy.

The hypothesis advanced by Davenport assumes new significance when viewed in the light of Symbionticism and the known responses of microörganisms to a continually changing environment. It is not only the metabolic products elaborated by living cells, however, that play the rôle of stimulators in cellular differentiation. The significance of iodine in relation to normal development and growth illustrates the dependence of some organisms upon chemicals derived from the environment.

CHAPTER X

Symbionticism and Organic Evolution

Various theories and hypotheses have been advanced at different times to explain the nature of the origin of life upon the earth. It is generally believed that certain chemicals, under the influence of unknown extrinsic factors, were combined in the formation of the first living matter. Some authors have attempted to suggest the nature of the substances supposed to have united under peculiar conditions of temperature, moisture and light. Some have held that "bions," or particles of living matter, were first transported to the earth from some other planet. Numerous attempts have been made artificially to produce living matter, but these have all ended in failure. While modern researches have extended our knowledge of living matter, we are far from possessing a full understanding of the nature of protoplasm. Until living matter can be produced artificially, one theory on the nature of its origin, perhaps, has as much value as another.

There is more or less agreement among biologists that the first or primordial life on the earth must have been of a bacterial nature. Osborn ('17) believes that "In the origin of life bacteria appear to lie halfway between our hypothetical chemical precellular stages and the chemistry and definite cell structure of the lowliest plants, or algae." The environmental conditions on the earth at the time of the origin of the first life, limit the nature of the first living organisms to such forms as were capable of existing on inorganic materials.

In their power of finding energy or food in a lifeless world the bacteria known as *prototrophic*, or "primitive feeders," are not

only the simplest known organisms, but it is possible that they represent the survival of a primordial stage of life chemistry. These bacteria derive both their energy and their nutrition directly from inorganic chemical compounds, such types were thus capable of living and flourishing on the lifeless earth even before the advent of continuous sunshine and long before the first chlorophyllic stage (algae) of the evolution of plant life. [Osborn.]

The next stage in organic evolution involved the production of *variations* in the primordial bacterial strains. In this connection, Osborn suggests a possible mechanism by which this could occur, on the basis of studies on *Nitroso monas* which lives on ammonium sulfate and produces nitrites, and *Nitrobacter* which lives symbiotically with it (bacteriological symbiosis). The *Nitrobacter* utilizes the nitrites and produces nitrates. It is reasonable to assume that early in this bacterial evolution chlorophyl-bearing bacteria or blue-green algae arose, perhaps by a process of bacterial fusion or under the influence of extrinsic factors. It must be emphasized again, that bacterial organisms are readily modified by environmental influences. *It is not known if these modifications are permanent, or under what circumstances they may become permanent.* On account of the importance of starch in the world today, it is thought that chlorophyl-bearing organisms arose very early in evolution, and hence from practically the beginning of life on the earth, chlorophyl-containing organisms have been the "food factories."

The next important stage in organic evolution witnessed the introduction of "organized cells"—the cellular animals and plants. The simplest representatives of both of these groups are composed of a single cell. The problem then resolves into a determination of the nature of the origin of the first true cells in evolution. (We are using the term "cell" to include only such units as have an organized

nucleus contained within a nuclear membrane. While the bacteria are often spoken of as "cells" we prefer, in agreement with many bacteriologists, not to call them "cells." Obviously this is chiefly a morphological distinction, but it also represents a distinct stage in organic evolution.)

A number of views have been advanced on the nature of the formation of the first cells. It is not clear from the literature, if a distinction between true cells and micro-organisms has been made in these theories. In some cases bacteria have been designated as cells. The theories and hypotheses that have been advanced, necessarily, have been speculative in character. At one time it was a matter of considerable controversy among biologists whether chromatin or protoplasm is the more ancient. "Boveri suggested that true cells arose through symbiosis between protoplasm and chromatin. . . . . Wilson believes that chromatin and protoplasm are coexistent in cells from the earliest known stages, in the bacteria and even probably in the ultra-microscopic forms" (Conklin).

If bacteria were the first forms of life on the earth, as we believe, then the one-celled plants and animals, necessarily, must have originated from the bacteria, if we are to accept the theory of organic evolution. A separate and distinct origin would be contrary to the principle of evolution, and it would mean nothing more nor less than a return to the principle of separate creations. As students of biology, we are bound by all the evidence that has ever been brought to light to uphold the principle of organic evolution. We may discard the view that bacteria were the primordial organisms on the earth, but that would be against the dictates of our better judgment. It then becomes necessary to attempt to explain the causes for the variations in bacteria that have led to the origin of one-celled plants and animals.

The question arises: Did these variations of bacteria

that gave rise to the protozoa and algae, originate as the result of environmental influences, or are they the result of some other factor. We have maintained, and the evidence submitted by many observers supports the contention, that bacteria are changed or modified by environmental influences. To what limits these modifications may proceed is not known. Most bacteria, perhaps, contain chromatin diffused in the bacterial body. Some of the higher bacteria appear to possess a more or less distinct nucleus. It is not known whether or not these are true nuclei. The evidence appears to favor their true nuclear character. Other bacteria contain granular masses within the bacterial body that have the staining properties of chromatin. The variations of chromatin material in the bacteria, from a more primitive diffused state to that in which it appears as a true nucleus, seems to indicate a gradual developmental process. It might appear that in such simple forms nothing but environmental influences could have brought about these variations. Such an hypotheses is further enhanced by the known fact that bacteria are so sensitive to environmental influences. Nevertheless, we venture to advance a different hypothesis of the nature of the mechanism whereby the one-celled plants and animals may have been derived from the bacteria.

It is not only conceivable that prototaxis was in operation in these early stages of evolution, but that this factor must have originated when life itself began. It is then logical and reasonable to assume that bacteria of different strains responded to positive prototaxis by fusing into a single mass or entity. In such a "fusion bacterium," at least two chromosome- and cytoplasm-complexes united to form an organism of an order higher than that of either component. It is also conceivable that further prototactic fusions took place between the more complex bacteria and the simpler ones. As the result of such fusion activities,

the chromatin increased, and perhaps became morphologically modified with each fusion, ultimately forming a definite body—the nucleus—surrounded by the cytoplasm.

Obviously, the formation of a definite nucleated cell did not produce a structure that became absolutely fixed and incapable of further prototactic activities. Subsequent "fusions" resulted in myriads of different kinds or species of one-celled organisms some of which may be in existence today. We also recognize in these plants and animals the 'partially fused' microörganisms (mitochondria) that appear to have been and undoubtedly still are the fundamental factor in the production of new forms.

It is well to consider again the modifications that bacteria exhibit when they become symbiotically associated with a cell. In the discussion of the early stages of evolution, we assumed that the bacteria "fused" to ultimately produce a nucleated cell. In such fusions, the identity of the original bacteria may have been completely lost. In the mitochondrial microörganism, a certain identity is retained by the microsymbiont, the mitochondria being present as definite bodies in the cytoplasm of the host cell. It must again be emphasized that microsymbionts may undergo even radical modifications. A number of bacterial symbionts retain their independence to the extent that they are readily cultivated in ordinary culture media. Others appear to develop fragility, and, perhaps become modified in other ways making them difficult to stain and to cultivate in artificial media. We have previously discussed the behavior of mitochondria, and mentioned the phenomenon in which they seemed to "dissolve" in the cytoplasm. We may again direct attention to the observations of Chambers, in which he apparently saw the mitochondria go into solution and again reappear as bodies in the cytoplasm. It appears probable *that in some instances the microsymbiont completely fuses with the host cell.* It

also appears probable that in some cases the fused microsymbiont may again resolve into a definite body.

Obviously, it is quite impossible with our present methods to determine the ultimate modifications that microsymbionts may undergo. Many factors undoubtedly are concerned in these responses, chief of which are the prototactic properties of the two symbionts involved. From the observations that have been made, we are justified in concluding that the modifications experienced by microsymbionts range all the way from an invisible response to those in which the microsymbiont completely fuses with the host cell. It is evident that we have no means of detecting the presence of a microsymbiont that has thus completely fused with the host cell.

To return to the question of bacterial fusion, we might ask: Is there any evidence to indicate that bacteria actually do fuse with each other? Although the researches made in this field are of rather recent date, and perhaps have not been fully verified, nevertheless, direct evidence has been submitted by Löhnis and Smith ('16), Löhnis ('21), Almquist ('22) and a few others that bacteria may exhibit the fusion phenomenon. Löhnis especially has investigated the behavior of bacteria, and has furnished much evidence in support of bacterial fusion not only from the literature, but from his own observations and experiments as well. The fusion phenomenon of bacteria has been called by Löhnis "symplasm."

In the life cycles of bacteria, a stage develops in which a number of individuals of a strain come together and produce a rosette. Presently, the morphologic identity of the bacteria is lost; the rosette resolves into an amorphous mass or body. This amorphous body is the "symplasm." Later in the course of the life cycle individual bacteria reform from the symplasm, and again take up their vegetative functions.

Symplasm in the life cycle of bacteria represents a temporary fusion stage somewhat akin to conjugation, but more intimate in character. Even in this process, we can account for a mechanism in bacterial variation that coincides with that embodied in Symbionticism. The possibility of foreign bacteria with harmonious prototactic properties being engulfed or "drawn into" a bacterial symplasm appears feasible. Such bacterial contact would allow for a modification of the individual bacteria after they had separated from the symplasm.

The conception that "bacterial fusions" have been the fundamental factor in bacterial evolution or variation, is necessarily hypothetical, but it is in harmony with the principle of Symbionticism, and it appears to the author that it offers a rational explanation with at least some "circumstantial evidence" to support it. To what extent environmental influences may enter into the process of bacterial evolution is difficult to analyze even in a speculative way. Certainly, if environmental influences can modify free-living bacteria and *produce permanent modifications*, then we are justified in assuming that they may also operate in the origin of higher organisms. However, it remains to be shown whether the modifications produced in bacteria by environmental factors are transitory or of a permanent nature. It is difficult to understand how environmental influences alone could, for example, bring about changes in a group of cells or a microörganism that would result in the production of an entirely new chemical product. On the basis of our experiences with chemical reactions, it is easy to understand how bacteria of different potentialities might combine to produce a new organism with properties differing from those of either component. Just as in chemical reactions a certain combination of extrinsic factors are necessary if the reaction is to take place, so it is safe to assume, that environmental factors

of a certain character or combination, may be essential for the development of intimate symbiotic complexes.

Pleomorphic responses of mitochondria to environmental influences have been shown by a number of investigators. The sensitive properties of mitochondria led Cowdry to say: "The central thought which underlies all the recent work on mitochondria in pathological conditions is the conviction that we now have at our disposal a new criterion of cell activity and cell injury. We do not know it or under stand it, but it has been proven over and over again in the last few months to be of great and surprising delicacy, for it responds (even before the nucleus) to injurious influences; and it has the rare merit of being cytoplasmic. We may expect environmental changes to act on it which would make no impression at all upon the nucleus." It appears highly improbable, however, that environmental forces by themselves are capable of producing permanent modifications. In the previous chapters we have suggested the possibility that environmental influences acting upon mitochondria may be responsible for hereditary variations, but we do not believe that such variations, by themselves, would be of evolutionary rank.

We are not in a position to deny the possible influence of environmental factors in the origin of species. It appears probable that they play the rôle of at least secondary factors. Certainly we recognize the definite influence of environmental factors in organic evolution. In that phase of evolution that we have called the "retention and destruction of species" we recognize the significance of environmental influences. We must also emphasize the probability that Symbionticism may be a factor in the "destruction of species," as well as in their origin. Symbionticism may lead to retrogressive as well as progressive modifications.

In the theories on the origin of species that have been

advanced in the past, attempts have been made to explain numerous specific variations in plants and particularly in animals. These explanations have not been favorably received among biologists. It is not necessary to review this extensive literature, as the well informed biologist is acquainted with the analyses and the criticisms that have been launched against them. The general reader who desires to learn more about these views and criticisms is referred to Kellog's "Darwinism Today" ('07), which gives a comprehensive and unbiased discussion of the subject.

In the evolution of higher organisms, the modifications of structure and function is ever increasing so that in the mammalia and in man the organism has become an extremely complex mechanism. The normal activity of a single tissue or organ is not only dependent upon its particular intrinsic organization, but as recently demonstrated, upon a number of extrinsic factors as well. Thus, it has come to be recognized in endocrinology that few, if any, of the organs of internal secretion perform their function independent of other organs in the system. The inter-relationship of various activities is well illustrated in the mechanism of carbohydrate utilization in the mammalian body. Starches are digested in the intestinal tract under the influence of digestive enzymes produced by gland cells. (The importance of symbiotic bacteria in the digestion of cellulose by ruminants was previously mentioned.) The cells of the body, unassisted, are not able to utilize the sugar produced in this digestion. It has been known for many years that a substance, a hormone, is produced in special cells located in the islands of Langerhans of the pancreas that is essential for sugar metabolism by the cells of the animal body. This hormone has been named "Insulin." Banting and Best ('22) succeeded in isolating this chemical substance and more recently Abel ('26) has obtained it in

crystalline form. Experimentation has shown that an excessive amount of insulin in the tissues of the body is, perhaps, as injurious to the organism as is an insufficient quantity. Epstein and Rosenthal ('25) have demonstrated that the quantity of active insulin present in the blood is regulated by the amount of pancreatic secretion (trypsin).[1] In turn, the pancreatic secretory output under normal conditions is governed by the food intake. In such a complex mechanism, we can appreciate the significance of *intrinsic* environmental factors, but it is difficult to conceive of *extrinsic* influences as having had a hand in its development.

It might seem futile to attempt an analysis of the origin of structures and variations in organisms in a purely hypothetical manner. However, we feel justified in directing attention to a correlation that supports the theory of Symbionticism. The essential factor in the carbohydrate-utilization-mechanism, discussed in the previous paragraph, involves the production of two substances by two different sets of cells. It appears significant that both of the substances essential to this mechanism may be produced by bacteria. The mechanism regulating the amount of secretion from the cells must be looked upon as resulting from an intrinsic adjustment. It appears logical to assume that the acquisition of the ability to elaborate these substances (diastase and insulin) was associated with microsymbiosis.

While a large number of investigators have claimed that various secretions in the animal body are the products of mitochondria, others have made claims to the contrary. It is evident that more intensive investigations must be made before we are in a position to say definitely that mito-

[1] Horning ('25a) has shown that mitochondria leave the pancreatic cells and enter the adjacent blood vessels as well as the excretory ducts. Horning maintains that the mitochondria transform into the zymogen granules or the secretory product (trypsin) of the pancreas cells.

chondria are or are not concerned with secretion. (See discussion, p. 44.) So also, a number of investigators have claimed that myofibrillae, the fibrillae of connective tissue, pigment granules, and other cellular products, arise from mitochondria, and still others claim that mitochondria are not involved in these activities. The relation of mitochondria to plast formation, on the other hand, appears to have been accepted by most biologists, even by those who oppose the claims made for mitochondrial activity in animal tissues. It is difficult to understand or appreciate the basis for such variations in interpretation, even on the assumed incompatible chemical constitution of mitochondria, for obviously, if mitochondria can transform or differentiate into plastids containing chlorophyl, there is no reason for assuming that they are incapable of differentiating into bodies with diverse properties. If all mitochondria are presumed to be alike in chemical constitution, then we should surely expect to find chlorophyl in all cells. On the contrary, it is quite evident that all mitochondria are not alike. Recent studies have shown that they are not all alike in a single cell.

We have indicated the method by which one-celled plants and animals might have originated from the bacteria. Obviously, the different species of protozoa and algae arose from symbiotic combinations of different strains of bacteria with the primordial cells. The algae deviated from the protozoa in having prototactic properties that attracted blue-green algal symbionts. One protozoan, at least, appears to possess prototactic properties that are somewhat akin to the algae. This form is *Euglena viridis* which contains chloroplasts. Bacterial symbionts (mitochondria) have also been demonstrated in the algae. There are many species of algae, and we are justified in assuming that the different species owe their differences to the bacterial symbionts (mitochondria) which they harbor. So also, in the

protozoa, the different species find a ready and reasonable explanation for their origin in Symbionticism. The ciliate and flagellate protozoa, apparently, acquired their special locomotor structures through symbiosis with ciliate and flagellate bacteria. We have previously mentioned that some investigators have claimed that cilia formation in the cells of higher organisms is associated with mitochondria. So also the origin of those protozoa having the ability to produce calcareous shells about their bodies, finds rational explanation in Symbionticism. Bacteria with the property of secreting calcareous materials are known.

One of the simplest metazoa (many-celled animals) is *Pandorina*, in which all the cells look alike. It consists merely of a colony of cells that do not separate, but remain attached. On the basis of Symbionticism, it is readily conceivable how such an organism may have arisen. It is rational to suppose that a one-celled animal acquired a microsymbiont of such properties that it modified the physical nature of the host cytoplasm, which was so altered that the cells after division remained together. This cohesive property of the protoplasm was a fundamental acquisition, for it has persisted in the development of all higher animals. Obviously, the extent to which this physical property (viscosity) is found, varies in different groups of cells.

There are some animals that are considered "regressive" organisms. One of the most widely-known groups of this character is the *Tunicata*. The adult organism exhibits a morphology of a lower order than the developmental or ontogenetic history would indicate. These organisms have aroused considerable interest among students of evolution, and various hypotheses have been advanced to explain this "retrograde" development. Presumably, the origin of these forms is attributable to the same set of factors responsible for the production of the progressive types. It

is reasonable to suppose that the acquisition of a particular "injurious" microsymbiont by the host organism led to degenerative responses. It is evident that the "destructive" influence does not operate on the germ plasm of the host, but becomes effective when the animal has reached a certain stage in its development and the microsymbiont has asserted its specific influence.

Paleontological researches, have shown that new species apparently have arisen suddenly. Furthermore, it seems that in certain geologic eras a large number of new species arose simultaneously. On the basis of these observations and the geologic indications, it has been suggested that "cataclysms" have occurred during various periods. It is quite certain that in the origin of species, large numbers of individuals of a given species were modified simultaneously. It is impossible to go back into geologic time and observe Symbionticism in operation. However, we find a possible clue as to the factors operating in Symbionticism, in the behavior of bacteria in some epidemic diseases. While we know some of the factors concerned in a number of disease epidemics (bubonic plague, cholera, typhoid), we have not been able to solve the mystery of the influenza pandemics that periodically have swept the world. This phenomenon illustrates the possibilities of bacterial behavior. The peculiar movements and appearances of these epidemics have been so puzzling that many pathologists believe that the organism associated with the disease is a modification of some form that is constantly present but harmless. Such a suggestion implies a response of the microörganism to some *unknown extrinsic influence.* Numerous attempts have been made to isolate a distinctive organism associated with the disease. Many claims of discovery have been made, but the concensus of opinion among pathologists appears to be that the organism associated with the disease is normally harmless and present

at all times. In this connection, Dr. I. S. Falk ('25) says: "A number and variety of microörganisms and filterable viruses have been incriminated, but none holds an unequivocal claim to a causative relationship . . . . . The etiology of epidemic influenza remains a riddle." Such opinions appear to indicate that extrinsic factors are responsible for a modification either in the microörganism or in large numbers of human individuals. The peculiar and rapid migration of the disease from East to West around the world, would also indicate that some extrinsic factor is concerned. A disease organism could hardly travel or migrate at the rate at which this disease spreads.

In discussing the relations of extrinsic factors to epidemiology, Falk says:

> The epidemiologist has been accustomed since the rise of the bacteriological fashion to scoff at the statements in older literature concerning the relations between epidemic prevalences and cosmic and telluric phenomena associated with sunlight, weather, rain, wind, earthquake, ground water-level, etc. The evidences which have been coming out of the studies on the vitamin deficiency diseases, notably rickets, in recent years, have begun to teach him that he may stay to pray. If recent findings in the value of heliotherapy, especially in relation to organic and cellular physiology, as is so clearly evidenced in rickets and in surgical tuberculosis, have demonstrated anything, they have shown that sunlight plays no inconsiderable rôle in the maintenance of the normal functioning of the organism. The far-reaching effects of sunlight are reflected in the physiology of parts remote from the surface tissues. These and other environmental factors may prove to be far more significant in the elucidation of certain problems in pathology than has until recently been suspected.

It would be futile to attempt to analyze, even in a hypothetical way, the factors concerned in the development of symbiotic complexes. We can only indicate the factors that influence bacterial behavior so far as we know them,

and particularly in connection with diseases. These factors are the forces that influence protoplasmic behavior, such as, temperature, light, moisture, pressure, electricity, and possibly gravity, solar and lunar forces. In connection with disease, and also symbiosis, we do not know whether the host or the microörganism, or both, are modified by these extrinsic influences. Perhaps, when we some day have acquired more specific data on meterological influences on living matter, we may then better understand the behavior of living organisms.

It is highly unsatisfactory, and it invites criticism, to delve into extensive hypothetical analyses and discussions. Nevertheless, it appears to be necessary to predicate scientific progress with speculation. These hypothetical analyses and discussions are not offered as evidence of the reality of Symbionticism, but entirely in the spirit of demonstrating the rationality and the feasibility of the principle of Symbionticism. The evidence for the operation of Symbionticism in the origin of species is to be found in those forms in which the microsymbiont still retains its identity. This was reviewed in the chapter on "Microsymbiosis," and it appears to be conclusive. Aside from this direct evidence, we have submitted what might be called "cricumstantial evidence" which supports the direct evidence. The bacterial nature of mitochondria indicates the universal operation of the principle of Symbionticism. The correlation of bacterial products and the products of specialized cells in plants and animals demonstrates the feasibility of the principle of Symbionticism in the origin of species.

Although there was no acceptable evidence of the bacterial nature of mitochondria at hand when Buchner ('20) wrote his extensive review on microsymbiosis, he, nevertheless, recognized its probable significance in the origin of species. The following quotation makes this clear.

If we refuse to create a completely new principle of structure of the cell through intracellular symbiosis, it does not necessarily follow that intimate symbiosis may not be the stimulus for the development of new animal forms. We have previously indicated such possibilities in the insects, but it is of the greatest importance that, as a result of the mixture of an animal with a symbiont, new biological or physiological possibilities arise. In the course of their operation, old characters may be intensified or repressed, the latter, for example, as it occurs in wing-formation in the female *Lampyris* in which the misplacing of the wings and the loss of flying were due to the acquisition of symbiotic luminous bacteria. Entirely new characters may arise in connection with the symbiosis. [Translation.]

Buchner, apparently, recognized *secondary* modifications due to Symbionticism, but he failed to recognize the *primary* modification associated with the microsymbiont. *The host responded to the acquisition of the microsymbiont by developing a light organ.* This response was a *primary* modification in the host, and of sufficient magnitude to produce a new species.

It will occur to the informed reader that there are many features of organic evolution that may not be explainable on the basis of Symbionticism. It would be difficult, for example, to attempt a rational explanation of the development of "mimicry" through Symbionticism. So far as I know, there never has been advanced a rational theory to explain this interesting biological phenomenon. It would appear that "design" enters into the development of mimicry; we are unable to recognize any such element in Symbionticism. If an element of design enters into organic evolution, the factors controlling this principle remain to be discovered.

In the Introduction to this book, we outlined three major or cardinal factors operating in organic evolution. These three factors are controlled by three cardinal princi-

ples: Symbionticism, the fundamental principle controlling the origin of species; Natural Selection, the chief principle controlling the retention and destruction of formed species; and an unknown principle responsible for progressive evolution. It appears evident that the three principles are closely intertwined or associated in the control of organic evolution; one principle, apparently, can not exist independent of the other two. It might appear that "progressive evolution" is but the result of the combined operations of Symbionticism and Natural Selection. When one reflects on the more or less gradual morphologic and physiologic perfection of living organisms leading up to man, it is difficult to conceive of this *progressive* evolution having been directed by Symbionticism and Natural Selection alone. While it is just as impossible to conceive of the nature of this unknown principle in the absence of data as it was to visualize Symbionticism before the data accumulated, nevertheless, it appears to the author that a distinct factor or set of factors control progressive evolution.

Organic evolution may be likened to a mammoth, creeping, kaleidoscopic procession which began to move when life first appeared upon the earth. In the beginning the procession was small. With the passing eons of time, there has been an ever increasing multitude, slowly, but steadily, moving forward. New forms have constantly joined the procession, and old forms have dropped out. We have not been able to look back into the distant past and learn from whence the procession started; we are not able to look forward into the future and predict where the procession means to go; we are only trying to analyze and determine the nature of the factors responsible for the kaleidoscopic nature of the procession as it appears today.

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